



6 December 1979

Public interest representation on research councils?

Who runs Britain's science? A simple question capable of an exceedingly complex answer, so widely is science dispersed across the scene. But for university scientists and many others in specialist laboratories there is a relatively simple answer: the research councils, which fund individual research projects, provide studentships and fellowships and operate a large number of major laboratories, many with an international reputation.

And who sits on the research councils and on the Advisory Board for the Research Councils, which coordinates their efforts and provides advice to the Secretary of State for Education and Science on how to spend £300 million annually? Distinguished scientists and medical men, a handful of industrial scientists and one or two farmers (on the Agricultural Research Council). Council members are appointed by the Secretary of State.

Now to the best of our knowledge members of the research councils fulfil their duties diligently, giving up time that they can often ill afford to attend meetings, understand complex issues and help to steer policy. We do not question the effort that they put in. What should be questioned is whether the composition of the councils is right for the 1980s, and whether there should not be a greater representation of what, for want of better words, may be called the public interest.

Before we are accused of mischievously allowing amateurs and meddlers to interfere with such delicate issues as who gets grants, it should be made clear that each research council has a complex structure of committees which handles such matters; council only concerns itself with broader issues. But these could profit by being understood and debated by people with an interest in science but who have not necessarily followed the research path.

There is no shortage of constituencies which could make valuable contributions. They could include trade-unionists, teachers, those in overseas development, politicians, science writers, lawyers, businessmen, bankers and doubtless many others. Since much of science is international, there should be no compelling reason why a foreigner (particularly from the EEC) could not be incorporated for some purposes — one or two other countries already do use foreign help in deciding science policy.

Of course scientists themselves would have to be protected against being swamped by non-scientists, but the model of the Genetic Manipulation Advisory Group, which satisfactorily incorporates several 'public interest' members, would bear study. One of the weaknesses of Britain is the lack of cross-communication between experts in different disciplines. Here is a chance for an experiment in trying to build up such cross links. □

Flying university in trouble

HIGHER education and scholarship in Poland recently acquired an unusual and innovative institution — the Society for Academic Courses, better known by its nickname of the 'Flying University'. This society arranges lectures, seminars and discussions to satisfy the needs particularly of young students given to questioning the information obtainable through conventional channels. Although a large fraction of the courses currently on offer concerns contemporary history and the social sciences, scientists are amongst the sponsors of the society, and a course is included on moral issues in biology. The society has received notable public support from physicist Janusz Groszkowski, former chairman of the Polish Academy of Sciences, who resigned his official posts in 1976 in protest against police interference in academic life.

The educational experiment has not gone entirely smoothly. The police had been regular visitors to houses where seminars are held, and several lecturers have been detained and fined on

various charges of disturbing the peace. Seminars have also been visited frequently by gangs of students intent on doing damage. This term only the inaugural session, by the sociologist Witold Bartoszewski and the physiologist Jan Kielanowski (a member of the official Polish Academy of Sciences) was given before the wreckers moved in. Two of those involved, Dr Bartoszewski, and Piotr Naimski, a biochemist, have each been fined 5000 zloty — the equivalent of an average month's academic salary — for their activities.

An international support committee has just been formed to provide encouragement for an independent intellectual life in Poland by the provision of material help, including books, and by the publication of research results. It is a cause that scientists who have a large amount of freedom to say and do what they wish may wish to contribute to.

The address is 11 Bevington Road, Oxford. □



NASA told to delay space mission with European scientists

A US congressional committee last week directed the National Aeronautics and Space Administration to impose a two-year delay on a joint solar research project with European scientists, the International Solar Polar Mission.

The House Appropriations Subcommittee responsible for NASA's budget said the delay was necessary because of difficulties faced in the development of the space shuttle, from which two spacecraft would be launched simultaneously to orbit the sun in opposite directions.

In addition, the subcommittee has told NASA that both the solar polar mission and the planned Project Galileo mission to Jupiter, which the agency has already suggested postponing from 1982 to 1984, should use modified Centaur rockets for the launch from the shuttle, rather than the 'inertial upper stage' (IUS) currently under development by the US Air Force.

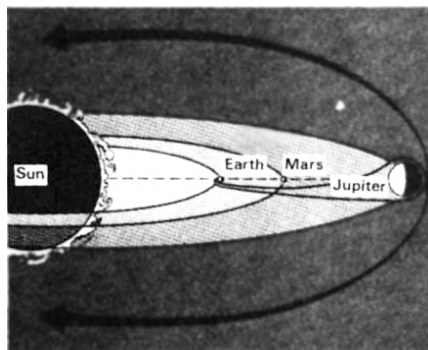
The instructions were contained in a letter to Dr Robert Frosch, administrator of NASA, from Appropriations Subcommittee chairman Mr Edward Boland. Two years ago Mr Boland tried to persuade the House of Representatives that Project Galileo should be killed entirely, and was only defeated after intensive lobbying by the space science community.

The legal status of the directive is unclear, since any change of plan would need to be endorsed by at least three other congressional committees. But with the space shuttle running into increasing delays and rising costs, space science projects have become vulnerable prey to budget-cutting politicians.

The solar-polar mission is a joint effort between NASA and the European Space Agency. The two spacecraft are currently scheduled to be launched in February 1983, and after a fly past Jupiter to enter polar orbits around the Sun in 1987.

Problems have arisen because delays in the shuttle development programme — now over a year behind schedule — have contributed to difficulties in guaranteeing that the IUS could be developed sufficiently fast to provide the thrust needed to launch the two spacecraft from the shuttle by the planned date.

Similar problems have affected the Galileo project, which is also planned by NASA to use the IUS, and for which NASA has now decided that there should be separate launches for the probe and the orbiter in 1984. Previously it had been



Planned trajectories of the ISPM spacecraft: a 2-year delay will add greater strain to ESA's badly stretched space science budget.

planned that both vehicles would be launched simultaneously by the IUS, but this could only have been done with a 1982 launch making use of the extra pull from the planet Mars.

Last summer NASA had rejected the Centaur as an alternative to the IUS, largely because of the extra development costs, which it calculated would have added an extra \$100 million to the cost of Galileo, whose predicted budget has already risen from \$450 million to \$675 million because of the time delay.

In his letter to Mr Frosch, however, Mr Boland says that his subcommittee will only let Galileo proceed 'if NASA develops a Centaur upper stage designed to fly a combined orbiter-probe Galileo mission in 1984', and that the solar-polar mission, also using the Centaur, must be put back to 1985.

ESA officials point out that, under their agreement with NASA, the US agency would have to bear any additional launch costs for the solar-polar mission. However they are seriously worried about the effects of a two-year delay, not only because of the extra costs of keeping scientific and technical teams together, but because it could initially mean the underutilisation of a small space science budget, as well as upsetting medium-term budget priorities.

NASA received more bad news last week, when the Office of Management and Budget rejected several proposals for new programme starts which the agency had submitted for possible inclusion in the President's budget request to Congress for the fiscal year 1981. The only new start to have received OMB's blessing is said to be the proposed launching of a gamma ray observatory. However the budget office has not supported NASA's request to start development work on a propulsion system necessary if a joint mission is to be carried out for encounters with Halley's Comet and the Comet Tempel 2 in 1985.

NASA officials are now hoping that the White House can be persuaded to support some of the projects deleted by OMB. In particular discussions are taking place over joint sponsorship between the Department of Defense, the Commerce Department, and NASA about establishing a national oceanic satellite system, a successor to the ill-fated SEASAT. □

UN split over science policy machinery

Member countries of the United Nations remain sharply divided over the type of internal machinery that the UN should set up to help implement decisions reached by the Conference on Science and Technology for Development (UNCSTD) held in Vienna in August.

At stake is the future of the Office of Science and Technology, currently the prime focus for science policy activities within the UN, but which many developing countries consider inappropriate, in its present form, to an expanded role.

Delegates attending the second committee of the UN General Assembly in New York last week, which is examining the conclusions of the conference, voiced unanimous approval for its decision to establish a new Intergovernmental Committee for Science and Technology for Development (IGC) open to all member states of the UN.

As in Vienna, however, opinion is divided over the nature of the secretariat which will service this committee. Many developed countries, and in particular the nine member countries of the European Economic Community, expressed their opposition to any new institutional machinery. They argue that the IGC could be adequately serviced by a strengthened department of International Economic and Social Affairs, under which OST now operates.

The Group of 77 however, which has been negotiating for the developing countries, has called for the abolition of the OST, and for its function and resources to be transferred to a new, independent secretariat, headed by an under-secretary general directly responsible to the Director-General for Development and International Economic Cooperation, Mr Kenneth Dadzie.

Mr Dadzie has himself offered a compromise solution which would create a new secretariat, but keep some resources within the Department of International Economic and Social Affairs. However many delegates feel that a clear division of responsibilities is necessary, and Mr Frank Da Costa, Secretary-General for the UNCSTD conference, has argued strongly that the Group of 77's proposal should be accepted.

After two days of open session, the second committee adjourned for informal discussions on the alternative proposals. A final decision is expected next week.

In addition to servicing the IGC, the new secretariat would be responsible both for co-ordinating science and technology activities within the UN system, and for providing support to the group of experts which the IGC will set up to study plans for a new financing system for science and technology for development. □

Recombinant DNA experiments to remain under safety guidelines

There will be no major removal of experiments using recombinant DNA techniques from safety guidelines laid down by the National Institutes of Health, writes **David Dickson**

Last week the director of NIH, Dr Donald Fredrickson, proposed in response to a recommendation from the Institute's Recombinant DNA Advisory Committee (RAC) that physical containment levels for virtually all experiments using the disabled K-12 strain of the bacterium *Escherichia Coli* should be reduced to the P1 level.

However he rejected the committee's suggestion that the experiments be formally exempted from the guidelines — and hence from NIH enforcement — proposing instead that they be placed in a special class which would still ensure that the containment requirements were met.

Dr Fredrickson's proposals were announced in the *Federal Register* last Friday (30 November) and follow a close scrutiny of the reasoning which led to the RAC's decision, taken by a vote of ten to four, with one abstention, at the committee's September meeting.

Rather than giving precise reasons for his decision, Dr Fredrickson lists the various events necessary for a serious accident to occur. In each case he quotes the opinion of scientists that the chance of such an occurrence is relatively low; and he points out that when a series of small probabilities are multiplied together, the chance of a sequence of events is "exceedingly low".

On the question of whether *E coli* could

cause an epidemic by passing from one person to another, for example, he quotes the conclusion of Dr Eugene Gangarosa, writing in a recent issue of the *Journal of Infectious Disease* that an epidemic "does not seem remotely possible".

Similarly in response to the question of whether *E coli* K-12 is pathogenic, Dr Fredrickson quotes Dr Sherwood Gorbach's conclusion that the disabled bacterium is intrinsically impaired in most, if not all, of a variety of virulence factors, all of which are required by a micro-organism to produce disease.

Taken together, suggests Dr Fredrickson, this evidence is sufficient to reduce containment levels for the bacterium to P1. However he says that he is not proposing to exempt the experiments, since the term 'exempt experiment' as currently used in the guidelines "should only be used for experiments for which no containment level is specified and for which no registration with the IBC is required".

As an alternative, Dr Fredrickson has proposed creating a special class within the guidelines for experiments using *E coli* K-12, except for otherwise exempt or prohibited experiments, or as otherwise specified. Experiments in this class would require P1 containment including a ban on

mouth pipetting and the requirement that all biological wastes are decontaminated.

"For these experiments no memorandum of understanding and agreement . . . need be submitted, nor is any registration with NIH necessary." However, suggests Dr Fredrickson, for these experiments, prior to their initiation, investigators must submit to their institutional biosafety committee a registration document that contains a description of:

- the source(s) of DNA,
- the nature of the inserted DNA sequences, and
- the hosts and vectors to be used.

The IBC will review all such proposals submitted by investigators, although such review will not be required prior to initiation of experiments. An exception, however, requiring both prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E coli* K-12 efficiently express any gene coding for a eukaryotic protein.

Elsewhere in this proposed revision to the guidelines, Dr Fredrickson repeats his proposal to introduce a new section covering in detail the way in which private companies can register their experiments with the NIH and submit them where necessary for approval to the RAC, but on a voluntary basis.

Dr Fredrickson says that a number of objections to this process have been received by NIH, claiming a voluntary system of regulation to be inadequate. However he also points out that the RAC voted by 11 to none, with four abstentions, in favour of these proposals, and says that he is therefore intending to include them in the revised guidelines. □

US legislators reject moratorium on nuclear power

In its first significant vote on nuclear power since the Three Mile Island accident last March, the US House of Representatives last week rejected by 254 votes to 135 a proposal that the Nuclear Regulatory Commission be required to impose a six-month moratorium on issuing construction permits for new nuclear power plants.

In a similar vote last July, the Senate had also rejected a moratorium proposed by Senator Edward Kennedy by 57 votes to 35. Since then, however, a national poll has shown 57% of those interviewed to favour such a move — and supporters of the moratorium were therefore disappointed not to have done better in the House vote, where they had hoped to pick up considerably more supporters.

The moratorium proposals have been the major focus of public debate since the TMI accident, much of it symbolic since the NRC has itself announced that it will not grant any new permits until it has absorbed the conclusions of the President's Commission of Inquiry, chaired by Dr John Kemeny.

In presenting the Commission's report last month, Dr Kemeny said that eight out of the 12 members of the Commission had favoured a moratorium of some kind; but no more than six — less than the needed majority — could agree on specific conditions under which a moratorium would be terminated.

Last week's proposal was put to the House by Representative Edward Markey, a Democrat from a working-class district of Boston, who argued that it was not meant to be a pro-nuclear or an anti-nuclear statement, but merely to signify that nuclear power should be made safe.

The TMI accident demonstrated that the regulatory framework had failed, and fundamental reforms were needed to make nuclear power safe. The amendment "would send a message loudly and clearly to the industry and the Nuclear Regulatory Commission that Congress will no longer tolerate any future corner-cutting in the area of nuclear safety", Mr Markey said.

Opponents of the moratorium, however, argued that it was unlikely to

have any significant effect — and that recent events in Iran had underlined the need for nuclear power to reduce dependence on foreign oil.

"The passage of the Markey amendment would be used by certain sectors of the media to dramatically harm public confidence in nuclear energy all across the world in the same way that they use the TMI accident to sensationalise and exaggerate nuclear hazards", said Mr Mike McCormack, chairman of the House Science Committee's Energy Subcommittee.

After the amendment had been defeated, Mr Markey said he still felt it was a good beginning "considering the 25 years of lobbying for nuclear energy and the very intensive effort by the utilities and the reactor manufacturers during the past few weeks".

In contrast Mr Carl Walske, President of the Atomic Industry Forum, said: "We think the House sent a very good message to the Ayatollah in voting it down". □

West Germany to propose big new accelerator

TODAY physicists at the Deutsches Elektronen-Synchrotron (DESY) hope to hear that they may go ahead with a proposal for a new two-stage, DM 700 million electron and proton collider to be built under a park near DESY in Hamburg.

The accelerator would be called HERA for 'Hadron Electron Ring Accelerator', and would provide collisions between 35 GeV electrons and 400 GeV protons. This would create the deepest probe yet of the three quarks which bind together to form the proton, and would take physicists one step forward to discovering whether the quark itself has structure.

If the West German ministry for research and technology (BMFT) and Hamburg City Council grant DESY leave to propose, HERA may be before the minister by January. Bjorn Wiik, who was behind a similar (but rejected) proposal at the European nuclear centre, CERN, has already progressed a long way with the design of HERA.

Furthermore, the BMFT is believed likely to turn a sympathetic ear to the project: the ministry is said to have a surplus of some DM 300 million of research money, and the Minister, Dr Volker Hauff, has frequently said that he wants to secure the future for DESY.

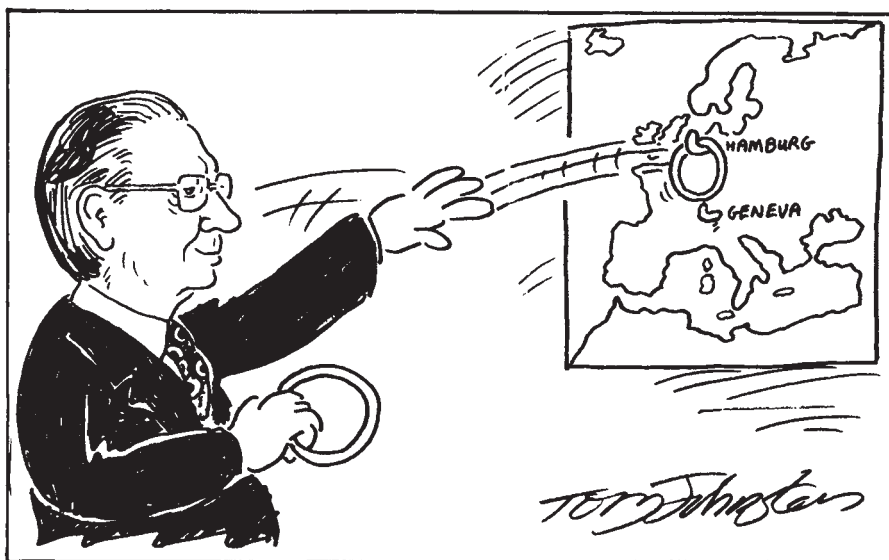
HERA is a project in two parts. The first is the building of a tunnel, deep in sand and the construction of an electron ring. "Surprisingly it is not easier to build a tunnel through sand than through rock" said a DESY spokesman. The tunnel would need concrete walls, magnifying the cost.

The cost of HERA, part one, would be DM 300-400 million. Part two involves the construction of a superconducting proton ring to accelerate protons to some 400 GeV; this would cost an equal amount.

It thus appears that the likely beginning of HERA will be a single electron ring of 35 GeV. This alone is of little use. But an electron ring one way round is a positron ring the other. So HERA will start by colliding electrons with positrons (their antiparticles) at a total energy of 70 GeV.

DESY insist that it would complement rather than compete with the large electron positron ring (LEP) that CERN hopes to build.

CERN's LEP would be designed with conventional magnets to reach an energy high enough to produce the Z^0 , a particle predicted by the Weinberg-Salam unification of the weak and electromagnetic interactions. (The predicted mass of the Z^0 is around 90 GeV.) With superconducting magnets (and accelerator cavities, an



Professor Schopper: a game of skill

unproven and complex technology) LEP would reach up to the 150 GeV or so needed to produce pairs of W^+ and W^- — the other prominent particles predicted by Weinberg-Salam.

HERA, on the other hand, without superconducting bending magnets or cavities, would not reach the Z^0 . Its role would be to look at the 'interference' between the photon and the Z^0 in electrons — positron interactions. (When they interact through electromagnetism, electrons and positrons exchange photons; through the weak interaction, almost completely masked at low energy by electromagnetic effects, they exchange Z^0 s). At HERA energies, the interference would be a maximum.

Superconducting magnets and cavities are not part of the plan for HERA's electron ring, says DESY; but if they do become available it would no doubt be tempting to DESY to raise HERA towards the Z^0 . A team at Karlsruhe have already had some success in superconducting cavity design.

Nevertheless the true goal of HERA is e^-p collisions, not e^-e^+ . "We don't want to take the Z^0 and the W s from CERN"

said the spokesman. "There is no war."

If HERA goes ahead, it would be likely to find international support, as have the present DESY electron rings DORIS and PETRA. The director of DESY, Professor Herwig Schopper, refused access to PETRA to groups composed purely of one nationality — including German. Thus researchers at DESY now include more non-German physicists than German. The French and British have the strongest representations at DESY; Japan, China and the US are also there. Italy, for the moment, is absent.

Schopper, however, may not remain director of DESY beyond the end of his term on 31 December 1980. On 1 January 1981, CERN itself needs a new Director-General, and Schopper is considered by many to be high on the list. CERN Council meets in two weeks — on 19 December — and the state of CERN-DESY relations at that moment may be assumed to be an important factor. DESY always raises the fear at CERN of competition for LEP, and the surreptitious announcement of HERA — within a closed meeting of the European Committee for Future Accelerators — has done little to dampen it. **Robert Walgate**

PETRA apprehensive of US rival

As PEP, the US rival to West Germany's PETRA electron-positron collider, approaches commissioning in the new year, PETRA physicists are concerned that PEP's likely higher event rate will eclipse the German machine.

PETRA has faced problems of resonances in the machine as it accelerates from its injection energy up to the experimental region. PEP will take injection at the experimental energy, and so avoids most of the resonances.

PETRA experiments have each collected "a few hundred" events in a year's operation. They have included evidence for the 'gluon' (the photon of the quark-quark force), and excited states of the upsilon

(which contains a beauty quark and its antiparticle), but 'truth' (the quark beyond and pairing with beauty) remains to be found, as does Z^0 -photon interference (which needs a 4-5 times higher event rate) and the details (*à la* charm) of the beauty spectrum.

In January, there are plans to boost PETRA to 36 GeV (total energy) by increasing the number of accelerating cavities from 32 to 60, but that will introduce yet more resonances. "If they get a factor five more events" said one DESY physicist this week, referring to PEP, "then we will be overtaken". This cloudy future may be one element encouraging DESY to make a strong play for HERA. □

Universities should opt for teaching or research

In his anniversary address to the Royal Society last week, Lord Todd, the Society's President, lamented the deteriorating health of Britain's science and argued for a restructuring of the university system. Here we publish extracts from his speech.

THE rapid growth of the university system in the 1960s brought about a vast expansion in tenured staff usually by the recruitment of relatively young men and women taken from the normal supply of university graduates and not invariably of the highest quality. Many, if not most, of these remain today at the same institutions and are likely to do so for perhaps another two decades under the tenure system.

A detailed study of chemistry departments in British universities has been made by Professor Colin Eaborn who summarised his findings thus: "At present only 7.8% of the staff of chemistry departments are below 35 years of age; the proportion will probably fall to about 4% in five years' time and rise to only about 9% in ten years time. The proportion below 40 years of age, now 26% will fall to about 12.5% in five years and to about 10% in ten years time. During those ten years the proportion of staff over 50 will rise from the present 28% to about 62%."

Viewing Europe as a whole, the European Science Foundation suggests that the chance for a junior research assistant to reach a permanent university appointment has gone down from about 70% during the 1960s to about 15% in this decade. The outlook is indeed bleak. Surprising though it may seem, the academic research profession has within a few years been transformed from one of the most mobile to one of the most static. This is particularly so in the United Kingdom, where the economic stagnation of recent years has all but stanchied the flow of academics in mid-career into other occupations. Many young scientists are seeking to retain their present precarious positions by hand-to-mouth grants because they fear that a change to another university or research institute might jeopardize their chance of obtaining a permanent appointment.

Several awkward, even painful, questions arise. Is it, for example, to continue to be taken as an article of faith that all established academics are capable of first-class research and that all students obtaining first- or upper-second-class honours degrees should be encouraged and provided with the wherewithal to pursue academic research? The answer to both questions must, I fear, be no. If we continue to believe that each department in every one of our present universities should have a substantial research school the strain on our financial resources may



Lord Todd: revise the dual support system

become intolerable.

The dual support system of the University Grants Committee and the Research Councils would ideally ensure that extra funds for the support of research were concentrated on those most likely to spend them well; as things now stand, however, while there is no reason to believe that really able young scientists with good projects to put forward will be denied temporary funding to initiate them, the prospects of their being given a sufficient measure of permanence to build up a centre of excellence in their institution is small.

It is not possible — and it may not even be desirable — that all departments of, say, chemistry or physiology should stand out for the high quality of their research. Some, for example, have too small a staff adequately to sustain undergraduate teaching and to supervise at the same time programmes of post-graduate work which all departments in all disciplines appear to regard as the essential breath of life. Need this requirement for large postgraduate programmes be universal?

It is a remarkable fact that in proportion to population there are more institutions awarding PhDs in physics in the UK than in the US. In the US we find many more institutions devoted to professional education and applied sciences and there are many distinguished universities whose reputation rests substantially on the quality of their basic teaching. Such diversity in our institutions should be encouraged. Why should there not be some differentiation between those who teach and those who pursue research?

Unfortunately the present machinery for the support of higher education and research in the United Kingdom was not devised for the encouragement of diversity. For all its undoubted virtues the University Grants Committee system does require that universities should compete within what is increasingly a common framework of objectives. So competition between them is almost always on familiar terms.

Preliminary (and anecdotal) evidence

suggests that in this competition Oxbridge and the older civic universities are winning out. If this be the case and the trend continues then we will end up with a hierarchy in which the universities lower in the pecking order will be trying to do the same things as the others but doing them with students who are not suited to them and would do better and become more useful citizens with a more vocational type of education.

If we are to change our present university pattern, the mechanisms by which we support research in academic institutions may also have to undergo some changes. Project grant applications will no longer be assessed simply on their merits alone without reference to the circumstances under which the research is to be carried out. Research projects in a given field will tend to be concentrated in one, or in only a very few, centres with considerably larger research groups than are usual today. It may well become necessary for the research councils to be more selective in the way in which postgraduate studentships are allocated to university departments; more radically, they may even have to think of making the grants to students of exceptional promise rather than to their potential supervisors. Another convention that may have to go is that every reasonably able PhD graduate can expect as of right to have two or three years of postdoctoral research during which he can establish a claim on a tenured research post.

The frequent frustration of this expectation is one of the saddest of the current symptoms of malaise in our university research. But the vigour of our research enterprise would surely suffer if all those concerned were now provided with a formal tenured career structure as many of them are now asking despite the current staff structure in our universities.

Unfortunately there is no quick or easy answer. A reduction in the university retiring age to 60 without any reduction in pension would undoubtedly speed up return to a normal age distribution, but would be expensive. Encouragement of voluntary retirement at 55 with generous financial compensation has also been suggested but would probably not be welcomed by more than a few individuals, and would also be costly.

If we accept that some universities will be much more devoted to vocational teaching and less to research than others, then only in a proportion of our universities need the situation be treated as urgent. These urgent cases could have a retiring age of 60 (with full pension) introduced if only temporarily so that a more normal flow of young academics could be reintroduced. I believe the overall cost would be tolerable.

● See page 552 for the legal position on short term contracts in the UK

news in brief

US scientists increase estimates of fluorocarbon survival rates:

Research workers with the National Oceanic and Atmospheric Administration have used air samples taken from a global network of monitoring stations to conclude that fluorocarbons emitted from aerosols and other sources survive for at least 30 years in the lower atmosphere, considerably longer than previous estimates of the minimum lifetime. The scientists say that the longer periods predicted when their sample data were applied to theoretical models of fluorocarbon behaviour "indicate that fluorocarbons do not undergo significant reductions in the lower atmosphere due to rain-out, fall-out, or chemical reactions, and thus are free to diffuse up into the stratosphere relatively undiminished".

However the scientists also found that the concentration of nitrous oxide fluctuates in the atmosphere, rather than demonstrating any steady growth. From this they have concluded that the potential threat to the ozone layer from gas given off as the result of massive applications of nitrogen fertiliser may not be as great as some have feared. "We had expected to see a steady upward trend in this chemical, corresponding with large increase in the nitrogen fertiliser, but no such trend was observed", said Mr Walter D Komhyr of NOAA's Geophysical Monitoring for Climate Change Programme in Boulder, Colorado.

US refuses to lift ban on pesticide: Despite pleas from representatives of southern states that the health hazards of the fire ant outweighed the hazards of a pesticide, Mirex, capable of controlling the ants, the US House of Representative last week refused to lift a ban imposed on the pesticide in 1977 when it was suspected of causing cancer. Some delegates argued that the known dangers of the ant, which invades the homes of the poor and can inflict a potentially dangerous bite, should take precedence over the unknown hazards of cancer. But they were outvoted by those who felt the need, in the words of representative E de la Garza of Texas, "to take the road of caution and not risk irreparable damage to people and the land".

At the same time, the House voted by 278 votes to 121 to give Congress the power to veto future pesticide regulations issued by the Environmental Protection Agency, although this has not been agreed by the Senate and is opposed by the White House.

MRC proposes less job security for researchers: The UK Medical Research Council has proposed eliminating its "tenure track" and will try to make all tenured jobs competitive on the open market. Under the proposed system a new researcher would be hired on a five year contract with the possibility for a three year renewal but there would be no automatic tenure procedure — individual researchers would compete with outsiders for the tenured jobs that became available. The Association of University Teachers, the trade union that represents the researchers is opposing the move. The present arrangement has five-year limited term contracts with a 75% chance of tenure at the end. It was opposed by many researchers when it was introduced in 1974 as a redundancy procedure that "permitted the MRC to lay off 25% of its annual cohort every year". The MRC was "not prepared to comment at the present time" on the new proposal.

University of Grenoble spectrometry lab occupied: The laboratory of physical spectrometry at the University of Grenoble has been occupied since 14 November by 15 technicians protesting a reduction in their pay of between 20% and 32%. The technicians, all highly skilled with 15-20 years of research experience, fell victim to a complex administrative decision on 1 November which prevents them from receiving pay from two sources — the university and the government — an arrangement that has existed for 20 years. The laboratory employs 130 researchers, technicians and administrators all of whom are in

support of the technicians. The lab's 85 physicists have lobbied the university administration and last Friday a one day full scale occupation of the administration building was held in an attempt to bring about negotiations. The matter is now under discussion.

New TB vaccine

needed in India: The BCG vaccine used world-wide against tuberculosis has been found to be ineffective in cases of lung TB in India. This is the finding of a major seven year study carried out by the Indian Council of Medical Research



Queuing for BCG vaccine in India: is it in vain? in conjunction with the World Health Organisation. The study has found, however, that the vaccine still affords protection against brain and bone TB. The revelations come at a time when the incidence of TB is showing a sharp upturn and the implications of the study extend to other Third World countries. There are an estimated 10 million TB victims in India with 2 million cases known to be infectious. Along with other Third World countries India is now confronted with the complex problem of evolving an alternative to the BCG. *B. Radhakrishna Rao.*

UK national anti-nuclear campaign launched: The first nationally coordinated extra-parliamentary anti-nuclear campaign in Europe was launched at a recent meeting of 600 activists in London. UK groups expected to ratify the campaign's constitution represent the full range of UK political opinion and include the Conservation Society, the Ecology Party, The Young Liberals, the Socialist Environment and Resources Association, the Socialist Workers Party, the National Union of Miners and local groups throughout the country. The campaign aims to reduce energy demand through conservation measures, to replace nuclear power with alternative energies and to guarantee employment during the changeover. Convenor Tony Webb said that the campaign would also focus on the withholding of information by the nuclear industry. "We have been calling for many months for the safety reports on both the AGR and the proposed PWR without success. Secrecy in these matters only confirms the widespread belief that the technology has not been designed for safety and is in fact extremely hazardous."

In the meantime a new UK study (*Decision Making for Energy Futures, A Case Study of the Windscale Inquiry*, Macmillan) has been published this week that is sharply critical of government policy on information. Calling for a freedom of information act authors argue that access to information, "a fundamental axiom of democracy", is a concept "alien to much twentieth century practice in the United Kingdom". And in a related development, the UK has refused to pay its share of £6m to fund an EEC research project to study the controlled meltdown of a Harrisburg type reactor at the Supersara pressurised water reactor in Northern Italy. Government officials say that the cost of the experiment does not justify the probable results.

US nuclear industry wins 1979 Doublespeak Award: The US National Council of Teachers of English has awarded its 1979 Doublespeak Award for the most appalling public use of the English language to the nuclear power group at Three Mile Island. Out-distancing a record number of nominees for the prize, the officials at Three Mile Island were cited for inventing "a whole lexicon of jargon and euphemisms" that played down the danger. Explosion became an "energetic disassembly", a fire "rapid oxidation" and a reactor accident a "normal aberration".

Berufsverbote continues in spite of resolutions

West German campaigners against *Berufsverbot* are planning to mark Human Rights Day, 10 December, with a petition to the Federal Chancellor, asking that the promised "liberalisation" of employment policy be implemented. **Vera Rich** talked to a leading member of the campaign about the effect of *Berufsverbot* on academic life.

BERUFSVERBOT is the denial of professional employment in the government service to persons of allegedly left-wing views. It affects West German universities and academic institutions because the funding of such bodies by the state means that their staff are "state employees" — in the same category as train drivers and postal clerks. Nevertheless, — and rather strangely in view of Germany's previous experience of political disruption of academic life during the 1930s — it was only relatively late in the anti-*Berufsverbot* campaign that certain universities and academic bodies began to take a stand on *Berufsverbot*.

the social-liberals are convinced that *Berufsverbote* must end — but it still hasn't stopped

In recent months, resolutions condemning *Berufsverbot* as a threat to academic liberty have been passed by the senates of the Universities of Oldenburg, Bremen, and Bielefeld, and the Deutsche Gesellschaft für Soziologie, amongst others. In the words of the Bielefeld protest (17 January 1979), the "autonomy of scientific work guaranteed by the German Federal Constitution forbids any attempt to exploit the scientist's status as a civil servant, to supervise his scientific activity by means of methods which are alien to science, or to derive restrictions on scientific activity from the loyalty and allegiance owed by the civil servant". The present practice of "screening and punishment for political beliefs", said the senate, "contradicts the liberal and democratic principles of our constitution," and, together with the administrative decrees on which it is based, "must be declared null and void".

Nevertheless, within a few weeks, Jan Prieue, selected by Bielefeld University as assistant professor of sociology was subjected to an *Anhorungsverfahren* (interrogation process) based on alleged left-wing activities more than five years previously.

Dr Andreas Dress, a leading campaigner against academic *Berufsverbot*, told *Nature* that it was precisely this screening process which was to have been "liberalised", but that "in fact almost nothing has changed. What we wanted was an end to the involvement of the *Verfassungsschutz* (Security Service) in job

placements". In his own *Land* (province) of North-Rhine-Westphalia, for example, applicants have to fill in a form giving all their addresses for the last five years. This list then goes on to the *Verfassungsschutz* computer, "proving," he said, "that the state government does not trust its citizens. But in a democracy, the state exists for the good of the citizens — and the Federal Republic of Germany is a democracy! This convinced the social-liberals that the practice must end".

"But it hasn't stopped," he added, "which is why we have organised the current protest. And even if it did officially stop, the personnel section could always pass on addresses to the *Verfassungsschutz*. Still, it would be something if the practice were stopped at least officially."

"We are not arguing about genuine security checks," he went on. "We accept that they have to exist. But security can go too far. One politician, for example, justified *Berufsverbot* on the railways by noting that, during the Nazi occupation of France, French resistance railway workers had been able to half the whole country's rail traffic!"

Within the universities, Dr Dress explained, there is no over-all policy of screening one faculty more closely than another — sociologists, say, more than mathematicians. Some institutions, however, seem more at risk than others. Thus in the Max-Planck Institute of Biology, Chemistry and Physics in Göttingen, screening procedures do not seem to have been applied.

On the other hand, certain universities — Bielefeld included — seem to have more than their fair share of *Berufsverbot* cases. This, explained Dr Dress, is a result of the more liberal and open-minded attitude of such universities to innovations in course structure and content. Potential victims of *Berufsverbot*, he said, are less likely to apply to highly traditional establishments, either because they feel they would have no chance of acceptance, or simply because that particular milieu does not attract them. He cited the example of Horst Eckhart Gross, a victim of *Berufsverbot* screening when he applied for a post at the University of Oldenburg. Previously, Gross had directed a Bielefeld research project on the non-academic job deployment of mathematicians.

Of university cases of *Berufsverbot*, said Dr Dress, some 80% related to alleged past or present membership of the Communist Party or the "Spartakus" Marxist student

union — both, he stressed, legal organisations. Some 10% of cases, mostly in Bavaria, relate to left-wing Social Democrats, and the remainder to miscellaneous left-wing groups. Formally, *Berufsverbot* should also cover far-right extremists, but according to Dr Dress such cases virtually never occur.

To date, Dr Dress knows of some 4,000 *Berufsverbot* interrogation procedures. These involve prolonged waiting, and, in some of the German *Länder*, such as Bavaria, those undergoing investigation are not allowed to use the services of a lawyer, while in North-Rhine-Westphalia, the right to legal assistance was won only after "a long struggle". Out of the 4,000 cases some 3,000 ended either with reinstatement in the desired post, or else in the applicant abandoning his claim and taking a job in the private sector. Some 1,000 clear cases of *Berufsverbot* remain on the campaigners' files. However, Dr Dress went on, academics have no "private sector" to go to.

The anti-*Berufsverbot* campaign assesses all alleged cases very carefully. Problems of student activists expelled for poor academic performance cannot strictly speaking be considered as *Berufsverbot*. Nor can the recent case — described by *Die Welt* as "grotesque" — of a biologist charged under the German Official Secrets Act for having given his East German brother-in-law an offprint of one of his openly published papers — this was a technical breach of the law. To the anti-*Berufsverbot* campaigners, it is important that all provocation be seen to come from the official side. "We are really only interested", said Dr Dress, "in cases where the authorities state in writing that the person is banned from employment on political grounds only. Then we can fight."

over 70 million DM has been spent in counter-propaganda.

The authorities, incidentally are not slow to take counter-measures against the damage done to their image abroad by the anti-*Berufsverbot* campaign. Dr Dress recalled that in April of this year, the Christian Democrats tabled a question in the Federal Parliament, demanding to know what the ruling Socialist-Liberal coalition had done to neutralise the influence of the campaigners. To date, according to the official reply, over 70 million DM has been spent in counter-propaganda, mostly among journalists in foreign countries. It is perhaps for this reason, Dr Dress concluded, that initiatives from the campaigners, such as the establishment last February of the "Heinrich Heine Fund" for victims of *Berufsverbot*, receive little publicity abroad. □

Brazil: running on alcohol

Brazil leads the world in the development of alcohol as a fuel for transport. But has it really found a way of appreciably reducing its dependence on oil? **Maurice Bazin reports.**

THE Brazilian government is plunging into a programme to convert all of its automotive transport to alcohol combustion within the next few years. The technical feasibility has already been proved, and several fleets of government vehicles already operate on pure ethanol.

The PROALCOOL programme was launched in 1975 in response to the world wide increase in oil prices, in an effort to contain Brazil's record US \$45 billion foreign debt. Domestic oil production accounts for only 16% of consumption, the rest being imported — almost exclusively from the Middle East — and refined in Brazil.

Ethanol (ethyl alcohol) is produced in Brazil from sugar extracted from sugar cane. Extensive cane fields dating back to colonial days cover the coastal north-east of the country; but these fields have very low yields. The landowners, referred to as 'colonels', reign over their peasants through armed 'captains'. Salaries remain below US \$2 a day and never cover the peasant's debt at the general store — which is owned by the colonel. Nowadays, 80% of sugar production comes from the more developed state of São Paulo to the south where agribusiness organisation prevails.

Alcohol production could currently satisfy one-third of Brazil's automotive needs if all private cars were run on pure alcohol. Brazil has in fact been using 'gasohol', petrol mixed with alcohol, since 1931, when the addition of 5% alcohol to petrol was made compulsory. Today, the mixture is not uniform throughout the country, being higher in the states with higher alcohol production, and reaching a maximum of 20% in São Paulo. Until 1975, the mixing proportions were not dictated by practical considerations, alcohol being dumped into petrol as an economic buffer for excess sugar production when international sugar prices dropped.

Some economic experts are apprehensive that the PROALCOOL programme will lose impetus if world sugar prices start rising again. They insist that to make the technological option irreversible and independent of the fluctuations of the

international commodity market, new distilleries should be built which operate directly from raw cane, bypassing sugar production and storage. The Institute of Sugar and Alcohol (ISA) which administers PROALCOOL has indeed approved the construction of 87 such new 'autonomous' distilleries. But it also approved the construction of 139 standard distilleries, which will double the present number of distilleries operating from sugar as an intermediary. This decision leaves some doubts as to the earnestness of the government's commitment to a fully independent alcohol program.

Alcohol production has increased dramatically in the past few years. In 1976-77, 600 million litres were produced; in 1977-78 production reached 1.5 billion litres and has risen to over 3.5 billion litres this year. The ISA announced that 'the sum of the already operating installations and of those projects already approved or under consideration should produce over 5 billion litres of alcohol. We can be assured of an overall production of 4 billion litres in 1980, which will guarantee the 20% level for the mixture of alcohol with gasoline'. The goal for 1985 is 10.5 billion litres.

One problem with this rapid increase in production is storage. Due to a miscalculation by ISA, storage capacity was found to be inadequate this spring. As a result, distilleries in São Paulo are threatened with having to halt operations, and Petrobras, the national company controlling the petroleum industry, is hastily renting out its petrol storage facilities to store the alcohol presently being produced. Furthermore, the government authorised the export of 500 million litres of alcohol, at a price double that of imported oil.

Up to 20% ethyl alcohol can be mixed with petrol without any engine modification, and the optimum mixture, which minimises fuel consumption, occurs with 15% alcohol. Brazil pioneered the study of alcohol engines in the 1920s. For the past ten years, the Brazilian Air Force Technical Institute has investigated the use of straight ethanol in modified automobile engines, and each of the foreign companies that control the Brazilian automobile industry (Volkswagen, Fiat, Ford and Chrysler) has developed new alcohol-powered engines for passenger cars.

Existing petrol engines are already routinely modified in São Paulo for the Volkswagen 'beetle' and the Brasília model. The changes are few, and currently cost about US \$350 per car. The work is done in standard engine overhaul shops approved by the Ministry of Industry and

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Refuelling on 'gasohol', a petrol-alcohol mixture used in Brazil since 1931

Commerce. Since alcohol has a higher vaporisation temperature than petrol, the incoming air must be preheated to at least 70°C to insure complete fuel vaporisation. This is usually achieved by passing the air through a jacket around the exhaust system, but other schemes using the engine oil in a preheating jacket are also being investigated. These engines will not start when cold, so a small auxiliary petrol reservoir must be installed to start the engine on pure petrol.

As well as testing the use of straight ethyl alcohol in modified automobile engines, the Air Force Technical Centre has engaged in a vast public opinion campaign. It organised several public demonstrations two years ago including a 'National Integration Tour' during which three test vehicles covered 8000 km in tropical regions. Car races, normally prohibited in Brazil, have been specially authorised to test new ethanol-powered models. Brazilian-built Fiats and Volkswagen sedans (Passat) were run continuously for two weeks, covering more than 100,000 km without problems. In these and other competitions, racing drivers showed enthusiasm for the engines, finding they could reach higher mean speeds than were possible with the equivalent petrol-powered models. They discovered experimentally that the best performances were obtained with an engine temperature 10–15°C cooler than the gasoline engines. This developed a higher torque, along with improved engine durability. The only technical problem not yet fully tested is corrosion.

No systematic study of pollution caused by ethanol combustion has been undertaken by official agencies. But it is clear that alcohol is both lead-free and sulphur-free to start with; while the lower engine operating temperature is liable to produce less polluting compounds.

But PROALCOOL pollution problems appear dramatically at the source. Distilleries are always built beside rivers,

Maurice Bazin is a science journalist specialising in Latin America. He is advising the Brazilian government on the teaching of physics.

into which they dump the stillage, or 'bottom slops', coming out of the first distillation column at a rate 12 times (by volume) that of the alcohol produced. The total output of stillage in Brazil is equivalent in biological oxygen demand to the untreated sewage produced by a city of 200 million inhabitants. Studies at the Copersucar research centre in São Paulo show that the high potassium content of stillage is responsible for killing both fish and aquatic flora. Although stillage can be converted into fertilizer or simply spread back on the fields after 'lagooing' for a while, this is hardly ever practised in Brazil, where distillery owners are not bound by any environmental regulation.

The early political commitment to use alcohol as a fuel has led Brazil to a definite technical lead. National pride ran high in October when Frank Press, President Carter's adviser on Science and Technology, who was touring Brazil in an alcohol-powered car, declared: "This is not an aid mission. Our visit is to initiate a mutually beneficial cooperation. Brazil is ahead of the whole world in the area of alcohol fuel, and the United States is interested in the PROALCOOL programme." At the same time the Brazilian Minister of Industry and Commerce, accompanied by the president of the Alcohol and Sugar Institute, was in Bagdad to sign an agreement of technical cooperation with Iraq which includes an offer of Brazilian technical know-how.

A recurrent theme among planners and politicians alike is the drive for technological independence through the use of those Brazilian technologies which are best established. "We have many raw materials from which we can extract alcohol," says Jair Carlos Melo, professor of Energy Resources at the Federal University of Minas Gerais in Belo Horizonte. "We should not stimulate national competition between them but rather select those which are immediately usable. This attitude will avoid our facing in the future the same problems with PROALCOOL which we now face with the nuclear energy programme: finding ourselves dependent upon foreign technology." This implies emphasis upon sugar cane, however, and reinforces the conservative agrarian social structure in the Brazilian countryside, and increases the power of the sugar 'colonels' and the owners of mills and distilleries.

Plans to diversify the sources of ethanol are being made, however, at least on an experimental basis, in the Ministry of Agriculture. Ethanol could be produced by the fermentation of wood. The cost of a pilot plant with a capacity of 100,000 litres per day is officially estimated at US \$20 million, "which is less than our daily expenditure for oil imports," says the Ministry's general secretary. But the plan is far from complete; the choice of a technology best-adapted to Brazilian wood will be made by a semi-private company

still to be incorporated.

The other highly promising alternative for alcohol production is 'mandioca', the plant from which comes tapioca. Mandioca has the great advantage over sugar cane that it can be grown in poor soil. Traditionally it has not been grown on large plantations by powerful landowners, like cane, but has remained the poor man's foodstuff. Only one experimental distillery operating from mandioca exists at present in Brazil. It was built by Petrobras in the State of Minas Gerais as a demonstration plant on an industrial scale, and produces 60,000 litres per day. Ten other projects have been approved by the government, several to be run by universities. One is also an attempt to decentralise energy production: the alcohol from mandioca will be used to run a turbine and produce electricity in the northern state of Ceará. Without specific government subsidies, it appears that private entrepreneurs do not dare as yet to invest in the construction of mandioca distilleries.

The development plan for the PROALCOOL programme relies upon the investment of US \$1.75 billion in five years. So far, a loan of \$1 billion has been obtained in London but the Brazilian Central Bank will free only \$350 million per year for the programme, keeping the balance in reserve. That sum is at present almost entirely devoted to financing new distilleries and modernising existing ones.

Some still have doubts

Vocal critics of the programme are found among economists and sociologists concerned with overall agricultural development. They point out that with presently existing technology sugar will be the only prime material for the production of alcohol during the next five years, and the present area under sugar cane must be more than doubled to meet the goals of the programme. Sugar cane demands fertile terrain and often special irrigation. Unless there is overall planning and coordination of agricultural production, easily available financial incentives for sugar cane planting will increase the social imbalance of Brazilian society. Sugar cane, now 'alcohol cane', could easily displace much-needed food crops and the regional imbalance and social tensions already characteristic of the Brazilian countryside will be exacerbated.

Trades unions have taken advantage of the combining liberalisation of the military regime to promote debates about the government's options for development. The Federation of Agricultural Workers of the State of São Paulo and the Distillery and Refinery Worker's Union are conducting "forums of the unconsulted", warning that the extension of the sugar cane agro-industry will "increase the inequalities in land use and ownership, in income distribution and in the distribution of the benefits of progress".

Even some state governments reject the

grandiose schemes which the federal government has planned for them. Thus the Agricultural Secretary of the state of Parana, immediate neighbour of São Paulo to the south, plans to authorise the construction of only 20 new distilleries rather than the 40 of the federal programme. "I reject the megalomania of the State of São Paulo", says Reinhold Stephanes, announcing that production per distillery would be limited to 120,000 litres of alcohol per day instead of 400,000 as in São Paulo. Five thousand hectares are necessary to produce 120,000 litres per day, and the regulations established in Parana demand that 25,000 hectares be reserved for other crops in districts where distilleries will be located.

The greatest doubt about the whole PROALCOOL programme concerns its ability to contribute to a real reduction in the outflow of dollars, or even to economise upon oil imports. During recent seminars throughout Brazil to consider the country's energy options, several engineering consulting firms have pointed out that the substitution of petrol fuel must be considered in a much broader context than has been done so far. Existing refineries produce fixed, and roughly equal, proportions of petrol, gasoil and diesel fuel. These proportions correspond closely to the consumption of each product. Alcohol, however, would substitute for only one of them. Refining schemes are quite rigid and any modification in the proportions of the final products would require enormous investments on the part of Petrobras.

To reduce its imports of crude oil, Brazil must therefore "wage a global struggle against the barrel", says Sergio Trindade, director of Promon's Technology Centre, a large consulting firm in Rio de Janeiro. In his presentations at national seminars, Trindade has pointed out that Brazil is at present totally dependent upon diesel fuel for its transport; more than 90% of goods being transported by lorry. Maritime transport accounts for less than 3%, although the country has a very long coastline and excellent harbours. The latter have traditionally been used exclusively for exporting cash crops (sugar, cocoa, and coffee) rather than for internal commerce. To reduce seriously Brazil's dependence on imported crude oil, control external debts and achieve what the government calls 'strategic independence', all three components of the barrel must be reduced at the same time. This means that the whole energy consumption pattern of the country must be altered. Small steps are not enough. Trindade would like to see an 'alcohols' programme get under way as part of national energy sources evaluation effort. This is the dominant theme of numerous discussions taking place at present throughout the country with the participation of ministers and scientists to elaborate a new 'Brazilian energy model'.

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Short term contracts: the legal status in Britain

British law on fixed term contracts is unclear. Basic questions, such as 'what is a fixed term contract?' and 'how effective is a waiver clause?' have still to receive satisfactory answers. **Julian Fulbrook** of the London School of Economics, traces the recent legal wrangles.

THE shabby treatment of short term contract workers continues to be a matter of concern. Although in the scientific and university context the exploitation of workers has never reached the depths of casual work problems experienced in the docks or building industry, the existing legal position is far from satisfactory.

Various estimates put the numbers involved at around 10,000 postdoctoral and other short term contract researchers in the United Kingdom. Of course, a strictly legal analysis ignores the wider general debate on tenure and temporary posts. And no one can ignore the desperate plight of highly-trained individuals who have hardly settled into a new position when they are required to forage for the next project, knowing that a new generation of younger researchers will be cheaper to employ. Mr Justice Kilner-Brown remarked in a recent case that the legal profession contains a similar log-jam between pupillage and a tenancy in chambers, but most would-be barristers are quickly snapped up by industry and the Civil Service. No such immediate alternative presents itself to scientists.

In considering the legal position of short term contract workers, it is important to note the threshold qualifications for Unfair Dismissal and Redundancy. The minimum period before a claim can be issued is now 52 weeks of employment for Unfair Dismissal and two years for Redundancy. It is therefore commonplace for a research worker to be fully in employment for a number of years, but at wholly different establishments for periods of months, and never build up an entitlement. The position is perhaps most acute for women who would wish to take up state maternity pay but are unable to stay in one institution for the requisite two years.

The usual legal questions, however, revolve around the nature of dismissal by expiry of a fixed term contract, and the potential waiver by a short term contract worker of his job security entitlements.

It would be an obvious loophole if an employer could evade the statutory protection given to employees against Unfair Dismissal by giving an endless series of short term contracts. The law since 1963, on the whole range of employment protection legislation, has therefore laid down certain rules to stop up this loophole. Unfortunately it can do little to help the worker who, through force of economic necessity, has to move through a variety

of employments; however, if his employers are 'associated', the continuity of rights may be maintained. Essentially, in relation to Unfair Dismissal or Redundancy, employment protection exists when any fixed term contract, of whatever length, comes to an end without renewal. This preliminary hurdle passed, the law then turns to the question of whether non-renewal was fair or unfair.

The waiver provision is more complex. To achieve an effective waiver, the contract of employment must be for a fixed term of two years or more. The statute insists on four requirements before the waiver ousts the jurisdiction of an industrial tribunal:

- The waiver must be in writing, in contrast to the general rules on employment law which give binding effect to oral terms.
- The written agreement must be made before the expiry of the fixed term contract
- The dismissal itself must consist only of the expiry of the fixed term contract without its being renewed. For example, if the worker is accused of misconduct or 'not being up to the job', then his remedy is under Unfair Dismissal and the waiver is not a bar to his claim.
- The final requirement has been the basis of confused litigation: the contract must be for a *fixed term* of two years or more.

But what precisely is a fixed term? The legislation is not entirely helpful. Section 49(4) of the Employment Protection Consolidation Act 1978, which gives an employee statutory minimum periods of notice, uses the concept of a *term certain*, whereas all the other statutory provisions refer to a *fixed term*. The significance of this is unclear, but in any event it has been brushed aside in the recent leading case of the Court of Appeal in *Dixon v. British Broadcasting Corporation* [1979] Industrial Cases Reports, page 281.

To understand the importance of the *Dixon* case it is necessary to recall the earlier decision of the Court of Appeal in *BBC v. Ioannou* [1975] ICR 267. Here Lord Denning had said that a fixed term was "one that cannot be unfixed by notice". In other words if there was an express term to allow either side to part company amicably before the stated term, then the contract was no longer for a fixed term. As notice provisions are practically universal in contracts of employment this had the effect of greatly reducing the scope of the exclusion provision. This may have been desirable in some respects, but the case had disastrous consequences overall for the

short term contract worker. As a notice provision rendered a 'fixed term contract' simply an ordinary contract, the worker could not claim that he had been dismissed through the expiry of a fixed term. And it would not be possible to qualify for Unfair Dismissal or Redundancy under the head of an ordinary straightforward dismissal, or even under a constructive dismissal, because of course in reality the contract had terminated by the 'effluxion of time'. The short term worker fell into a limbo.

Various Industrial Tribunals and the Employment Appeal Tribunal tried valiantly to avoid this injustice. Now in *Dixon*, the Court of Appeal declares that they were wrong in *Ioannou*. Lord Denning admits engagingly that he admired the ingenuity by which the tribunals were evading his previous definition of a fixed term to achieve a 'sensible result'. He noted the criticisms of many commentators that an employer could always evade the Act by inserting a simple clause 'determinable by one week's notice', and he declared that this could not have been the intention of Parliament.

The details of *Dixon* were that the BBC employed two men in Manchester as 'house services attendants'; Lord Denning thought that this was "a grandiloquent way of describing a commissionaire or a porter". Both were employed under fixed term contracts which, after several extensions, were due to expire "unless previously determined by one week's prior notice in writing on either side". Each employee had signed the extensions which always included the notice provisions. The final fixed term was for only four weeks, but the BBC disputed strenuously that it was for a 'fixed term' on the basis of *Ioannou*. To illustrate the see-saw nature of litigation, it should be noted that the Industrial Tribunal decision followed *Ioannou*, the Employment Appeal Tribunal allowed an appeal distinguishing *Ioannou*, and the Court of Appeal dismissed the BBC's appeal and overruled part of *Ioannou*.

The upshot of the Court of Appeal decision is that a fixed term contract may nonetheless have within it the possibility of termination by notice and yet remain a fixed term contract in law. *Dixon* has therefore resuscitated a number of Originating Applications which, having been earlier knocked out on jurisdictional grounds, are now able to proceed before the Industrial Tribunals.

Paradoxically, the Court of Appeal has increased the importance of the waiver provision. The original rationale behind the redundancy exclusion was explained by Professor Grunfeld, Professor of Law, London School of Economics, as being that "an employee taken on for a fixed period knows from the beginning that his job ends on a certain date . . . The fixed term employee knows what is to come and is therefore in a position to plan ahead for himself". But such a view cannot tenably be expanded to cover an exclusion from Unfair Dismissal. The law at present provides for fair dismissal in relation to lacklustre performance in a probationary period, and the continuance of the arbitrary two-year fixed term rule operates merely as a loophole for an employer to evade the Act.

It is unrealistic to imagine that research workers can individually refuse the imposition of a waiver when there may be hundreds of other candidates willing to take up the post. Of course the practical solution is through collective negotiation

to abolish the waiver, but given the lack of unionisation and the ease of importing new research workers, this can only be a long-term aim. Meanwhile, even where a waiver is not utilised, employers mould their contracts to the law; a favourite device is the 21-month contract, which creates the problem of finance over the summer before the start of a new academic post.

An important question arises over multiple contracts: is it possible to amalgamate the cumulative period of a series of fixed term contracts to achieve a fixed term of two years? In *Ioannou* the Court of Appeal, in a part of the judgement that has not been overturned, held that it is only the 'final' contract which has to be considered. Only where this final contract is for a fixed term of two years or more can there be a valid waiver. Although lawyers would normally draw a distinction here between a 'renewal' or a 're-engagement', Lord Denning regarded this as "too fine a distinction for ordinary mortals". His approach was followed in the Employment Appeal Tribunal decision of *Open*

University v. Triesman [1978] ICR 524, where the employee was offered a post as a lecturer for a fixed period of 18 months.

However, this 'straightforward' view has strange consequences. If an employee starts with a 21-month fixed term contract and is therefore unable to make a waiver, but then goes on to a two year renewal with a waiver, he will be unable to claim a redundancy payment. The first period will be blotted out, and no claim is possible.

This and many other legal puzzles leave the law in an uncertain state. There is still the faint chance of an appeal in *Dixon* to the House of Lords changing the law all over again, or even the possibility of major legislative change by the present government which has as yet given no precise indications as to the parameters of planned amending statutes. The only certainty is that the present law is in urgent need of an overhaul to resolve the waiver dilemma. Although this of course will do nothing to change the demand for short term contract workers, it may prevent some of the anxieties suffered by them. □

THE subject of meat eating is one that entrances gourmets, repels vegetarians, and attracts anthropologists. It has fascinated physiologists ever since the experiments on the fistulated stomach of Alexis St Martin by William Beaumont.

Something new happened recently: it started in a restaurant in Washington that was serving Pennsylvania rattlesnake meat to its patrons, thus reversing the balance of nature in which snakes bite hominids. Incredible as this would have seemed even as recently as 30 years ago, this venomous serpent is protected as an endangered species. An employee of the US Fish and Wildlife Service wrote a letter to the restaurateur, tactfully pointing out the act of trespass, whereupon the restaurant switched to Texas rattlesnakes for serving to its herpetophagic patrons.

All seemed well at first — except for the Texas snakes — but perhaps a conservationist intervention will soon be organised on their behalf. However, the news leaked out, whereupon the offending civil servant was fired by order of the Secretary of the Interior. A thunderous protest against the firing was mounted by lovers of Pennsylvania rattlesnakes and haters of dictatorial cabinet members, so that the Secretary hastily re-hired the snake-protector, bowing to the uproar from Congress, and from environmentalists, who are still seething over the betrayal of the snail darter.

It was time, I thought, for some general comments on meat eating, a mode of sustenance that involves a complex pattern of approvals and taboos. Probably none of us is without prejudice, distaste or downright rejection for some forms of meat that are relished by ethnic

groups to which we do not belong. Nutritionally speaking, the proteins of various meats are quite similar in amino acid content, and are superior in this respect to vegetable proteins, but this does not affect their acceptance or rejection.

Avoidance of beef in India is one of the

Carnivorous practices



THOMAS H. JUKES

best-known taboos. One theory is that it originated when Brahmins renounced cattle sacrifice to improve their position in political rivalry with the Buddhists. The shunning of pork is centered in the Middle East, and the feelings of rejection can be intense enough that martyrs have chosen death rather than submitting to being forced to eat pork.

It is often firmly asserted that the Jewish ban on pork originated as a protective measure against trichinosis that was based on intuitive and shrewd trial-and-error observations. This is contradicted by Simoons, F.J. *Eat Not This Flesh* (University of Wisconsin Press, Madison, 1961), who points out that *Trichinella* was unknown until the nineteenth century and was considered to be a harmless organism for 25 years after its discovery. It is unlikely that spoilage of pork by heat is the reason for the rejection. Temperatures in the Middle East are no higher than in many countries such as Southeast Asia, where pork has been a prized food for many centuries.

The evaluation of other possible reasons for the ban, such as distaste for the uncleanness of pigs, is complicated by the fact that Jewish laws also banned the eating of 30 kinds of animal including some comparatively tidy creatures, such as snakes. Chickens, however, are on the approved menu, even though their eating habits are at least as unsanitary as those of pigs. Americans and Western Europeans, who avidly swallow live oysters, would recoil from eating dog, which is relished in Central Africa, China and Southeast Asia.

The eating of frogs and snails was formerly used to whip up feelings of xenophobic prejudice in England against the French. In riposte, the English were called 'limeys', but it seems difficult to arouse antipathies based on the consumption of fruit, and, in any case, limes prevent scurvy.

The new approach to the avoidance of foods, including meat, and especially beef, is based on arousing the fear of cancer. I shall discuss this, and more meat-eating matters next time.

correspondence

Researchers Information Exchange

SIR, — Neither your editorial *Time for a policy on scientists' jobs* (1 November, page 1) nor the report by Joe Schwartz on *Portrait of the out-of-work scientist* (1 November, page 7) deserve to pass unnoticed. It is high time that the maximum of serious attention was given to the undoubted crisis which has developed in medical research and in research in other fields. The case of Dr Ann Simmonds and the Purine Laboratory at Guy's Hospital is only the tip of an uncompromising iceberg, that in the not too distant future is certain to confirm at last the United Kingdom's position as a third rate economic and scientific power, with a complement of first rate scientists, and scientific opportunities going to waste. Now is the time to ask "How long is it?" until that future comes to pass.

Some months ago the Association of Researchers in Medical Science committee received a very stony answer from the Department of Education and Science when they entered a plea to the Secretary of State to initiate a major inquiry into the scientific research sector (in a time of constraint and crisis), with particular reference to the conditions of employment of scientists and support staffs. In the meantime researchers' organisations, trades unions and campus opinion has been mobilised to publicise the plight of many researchers and scientists (in all disciplines).

The need to act in order to protect and develop conditions of work now seems a necessary distraction that research workers must pursue for their own survival and for our scientific future.

Government complacency and indifference in these matters is no longer defensible. In fact, some gesture from that quarter might redeem the situation before it gets really out of hand.

May I, through your columns, appeal to researchers and scientists to press energetically for a major review of the whole research and development sector, by the combined efforts of government, scientific and professional agencies. If our current economic straits do not allow the full panoply of review, at least a start must be made to identify problems and solutions so that scientific research policy can once again be adequate and informed. If action is not initiated soon we must accept irretrievable losses and delays to scientific progress, and technological and social applications. Can we bear to estimate our regret in five or ten years time by not having taken a positive stance now?

In the immediate future researchers and scientists must take steps to collaborate and coordinate their efforts in what is likely to become a bitter political battle if no firm action to seek just remedies is taken.

To this end a group of researchers (including myself and Dr Simmonds) have already established and gathered support for a *Researchers Information Exchange* to collect, analyse and distribute information on all aspects of research and development, relating to employment and conditions of work, and in the broader area of organisation, cooperation, funding, planning and policy-making. A recent meeting of RIE participants at Leicester University voiced the need for unified policies in the field of research, and it is to this end

that readers and colleagues are urged to lend their support to this venture as a first stage in self-help to ensure the continued existence of a viable scientific community in this country.

Yours faithfully,

STEPHEN ROBERTS
Loughborough University, Leicestershire, UK.

Life-time of research for minority only

SIR, — You are right to say (1 November, page 1) that hard thinking is needed about the nature of scientific careers. As I have argued elsewhere, however (*New Scientist*, 17 May 1973) one of the foundations for such thinking must be that research alone cannot provide a life-time career for any but a minority. Until this unpleasant truth is more widely recognised, we shall continue to have the waste and personal tragedy which you illustrate in the same issue as your leader.

Yours faithfully,

D.W. BUDWORTH
London W4, UK.

Alexander Lerner: a call for action

SIR, — We are extremely disturbed about the case of the distinguished Soviet scientist, Professor Alexander Lerner, who for eight years has been incurring the displeasure of the Soviet authorities because he applied for permission to emigrate to Israel. Professor Lerner was Director of the Department of Large Scale Systems at the Institute of Control Sciences, and among his publications is a well known text book, *Fundamentals of Cybernetics*, which has appeared in many languages.

Following his application for a visa he was dismissed from his post and the Communist Party and forbidden to teach any students; expelled from the Editorial Board of the journal *Automation and Telemechanics*; removed from the public office of Chairman of the Scientific and Technical Committee of the University of Moscow. An attempt was also made to remove him from the post of local deputy Chairmanship of the Committee of the International Federation on Automation Administration which was rejected by the Federation.

After selling 40,000 copies, his book *The Beginning of Cybernetics* was taken out of production and his contract for this publication cancelled because of "mistakes in the manuscript", although a translation was published in many countries and it was recommended for the Competition of Best Books in 1967. The book *Optimal Heating of Metals* of which he was the scientific editor and one of the authors, was published in 1972 by the Metallurgy publishing house without his name being mentioned and without any reference to his works which are of fundamental value in this sphere. He has been prevented from attending conferences both within his own country and abroad.

Professor Lerner's daughter received a mathematics degree at the age of 15. In 1974 she and her husband were allowed to emigrate to Israel where they now live with their two children. Professor Lerner who is 66 years old has never seen his youngest grandchild.

We are endeavouring to ameliorate the fate of this man who is isolated from his scientific

colleagues and his work. His many interests clearly lie within those fields covered by the International Federation of Automatic Control (IFAC). We hope that these international scientific bodies such as IFAC will show they have a clear duty to defend the rights of scientists working in their subject area. We therefore publicly call upon the IFAC Executive Council and National Member Organisations to consider seriously the fulfilment of their obligations in the case of Professor Lerner.

Yours faithfully,

PETER ARCHER DAVID SHEPPARD
for the parliamentary contingent

NEVILL MOTT A.H. VANDEN HEUVEL
for the scientific contingent

The Lerner Circle, London NW3, UK.

European ingenuity in particle physics

SIR, — We write to correct some of the more grossly misleading impressions generated by the careless journalism of your article entitled "Can Yankee ingenuity keep US physics on the ball?" (1 November, page 2).

The reference to PEP as the machine "expected to be the first to discover the Z⁰" is obviously, from its context, a mistake for the antiproton-proton (pp) collider now under construction at CERN. This machine is made possible by European ingenuity displayed in the development of beam 'cooling' techniques — stochastic cooling was invented at CERN and electron cooling at Novosibirsk in the USSR. The feasibility of cooling p beams has been demonstrated at CERN and the pp collider project quickly launched to use the SPS ring as a store of both p and p. The similar project at Fermilab which you mention is totally dependent upon these techniques.

The method proposed by Richter to achieve collisions of 40 to 50 GeV electrons and positrons is indeed relatively cheap as it depends upon the existence of an expensive two mile long accelerator at SLAC, built in the late 1960s and the only one of its kind in the world. We applaud the ingenuity of this proposal and hope that the very formidable technical problems can be overcome.

However, even if successful, this device cannot attempt the fundamental research for which LEP is deliberately designed; that is, not only to make a complete study of Z⁰ physics but to allow the most searching investigation of the weak, electromagnetic and strong interactions, performing vital tests of the gauge theory approach — such as the one due to Salam and Weinberg — which promises a unified understanding of these forces. These objectives require positron and electron energies approaching 100 GeV, not attainable at SLAC.

The last decade has been a time of dramatic advance in our knowledge and understanding of fundamental physical processes and we welcome the enterprise and ingenuity being exercised by physicists and engineers, both in Europe and the US, to bring nearer the crucial tests so eagerly awaited.

Yours faithfully,

R. J. CASHMORE
J. H. MULVEY
Department of Nuclear Physics, Oxford, UK.

news and views

Differential attack

from Robert W. Cahn

THE essentially empirical science of chemical etching, used as a routine tool by the material scientist to reveal the microstructure of mineral samples, has developed in some unexpectedly sophisticated ways. One is the recent development of a thin-film ultramicrosieve (Kitada *J. mater. Sci.* **14**, 2765; 1979), the latest in a line of filters whose unlikely origins lie in an observation made years ago that chemical etching could reveal the damage tracks left by the passage of nuclear fission fragments through thin mica sheets.

This story begins in 1961, when R.M. Walker and P.B. Price at the Central General Electric Research Laboratory in Schenectady discovered that these damage tracks were revealed by chemical etching which pierced minute, uniformly-sized holes through the mica. Other minerals were soon found to behave similarly, and other forms of radiation found sometimes to yield etchable tracks. Walker and Price, aided by R.L. Fleischer, over the next few years applied this differential etching method to many problems in geochronology, planetary physics (the study of meteorites in particular), palaeontology and nuclear physics. The field has become large enough to achieve that ultimate accolade, a journal of its own.

The early mica work came to the attention of S.H. Seal, a clinical cancer researcher at the Sloan-Kettering Institute who needed an ultrafilter to hold back cancer cells from suspension in blood; nothing he had tried would do the trick. Price's irradiated and etched mica foils were just right (the holes were all 3–4 μm across) but the foils were too fragile. At this point, Fleischer found that a polycarbonate polymer could be substituted for mica and had better mechanical properties. Thus began the manufacture of GE's 'Nucleopore' biological filters, which, among other uses have a role in cancer diagnosis. The filters were described by Fleischer, Price and Symes in *Science* (**143**, 249; 1964), and the steps in this weird progression from fission damage research to clinical instrumentation are traced, step by step, in that excellent survey, *Applied Science and*

Technological Progress (313, NAS, Washington, 1967).

This case-history has evidently influenced subsequent research at GE. For instance, W. Desorbo and H.E. Cline, who were investigating directionally solidified eutectics, consisting of parallel metallic rods in a matrix, with a view to their use in jet engine turbine blades, found that differential etching left the rods protruding clear of the matrix. Porous metallic replicas made from such etched samples performed neatly as microsieves (*J. appl. Phys.* **41**, 2099; 1970).

Filters with holes a few microns across are considered coarse nowadays (too coarse, for example, for the isolation of airborne smokes), and a new approach has now shown how to make filters with sub-micron apertures. M. Kitada (*J. mater. Sci. op. cit.*) co-evaporated gold and germanium in vacuum onto sodium chloride crystals. Films 50–500 nm thick were evaporated, at or near the eutectic composition of 12 at% Ge. Germanium was deposited as round grains 20–250 nm across, depending on alloy composition and substrate temperature. The films were floated off in water and attacked by an iodated mixed-acid etchant, which selectively removed the more reactive germanium. Since the germanium grains grow in columnar fashion through the film thickness, removal of germanium leaves minute pores right through the residual gold films and the result is a thin-film ultramicrosieve.

Another form of selective attack which is not only technologically valuable but scientifically intriguing, is the vertical etching of silicon. This technique, which for several years has been quietly developed within the semiconductor industry, is now beginning to find applications in other scientific fields. An excellent account of the technique, its scientific basis and recent very various applications has just been published by D.L. Kendall (*A. Rev. Mater. Sci.* **9**, 373; 1979) who regards it as a 'whole new way of structuring and using a solid material'. The essential fact is that extremely narrow, deep grooves with depth-to-width ratios exceeding 100:1 can be etched in silicon. It is only necessary to start with a monocrystal slice accurately

parallel to (110), and to prepare an oxide surface mask with narrow gaps precisely parallel to the (110)/(1 $\bar{1}$ 1) intersection. Etching is very much faster in the [110] than in the [1 $\bar{1}$ 1] direction, because the newly formed (1 $\bar{1}$ 1) plane is quickly covered with an oxide film which severely hinders access of etchant (usually an aqueous solution of potassium hydroxide used at 80 °C), whereas other planes are not thus affected. Any divergence of the surface slit from correct alignment enhances the rate of etching of the sides of the groove.

Whereas it is easy to etch deep ultra-narrow grooves, it is not possible to etch round holes. A regular array of exact 5 μm -square holes has been fabricated by etching two populations of grooves, mutually inclined at 70°, from opposite sides of a (110) slice until they just meet (Bean, *IEEE Trans. ED-25*, 1185; 1978). Other uses include deeply etched solar energy absorbers (effectively thin-layer blackbodies), an elaborate high-area Si/SiO₂ capacitor only about 1 mm square, diffraction gratings efficient for high-order optical spectra and an infrared reflection polariser. The most subtle application is a device for ejecting ink-drops between closely spaced pairs of electrified p/n junctions to charge the drops, which can subsequently be electrically steered to form a printed image. This has cleared the way to computer-controlled printing without moving parts.

A variant on the sieve theme is a selective-ion device for separating ions such as Cu⁺, which diffuse very fast in silicon. A thin-walled meander is formed in a silicon slice by etching offset parallel grooves from the two sides; copper will pass selectively through such a silicon membrane, much as hydrogen passes selectively through palladium. If the membrane is oxidised by anodising in hydrofluoric acid, the oxide has pores which can be as small as 1 nm in diameter. These can be used to separate molecules of different sizes: larger pores, for example, can serve as virus filters (Watanabe *et al. J. Electrochem. Soc.* **122**, 1351; 1975; Gregor & Gregor *Sci. Am.* **239**, 88; 1978). □

Robert W. Cahn is Professor of Materials Science in the University of Sussex.

Probing eukaryotic gene control

from Ron McKay

FOR two decades after the structure of double-stranded DNA had been determined, the difficulty of either establishing or manipulating a DNA sequence made even a descriptive approach to DNA-protein interaction difficult. The recent advances in DNA chemistry have been followed by improved methods of studying the association of DNA with protein and these techniques have provided valuable insights into such interactions as that between *lac* repressor and its operator DNA, the paradigm in this field, and those between ribosomal proteins and RNA. At a different level however, the most striking conclusion of a recent meeting* on this topic was that prokaryote-based rules of genetic organisation cannot completely explain eukaryotic gene control.

A brown box

Any functional definition of a gene must include statements covering the mechanisms of transcription, translation, replication and recombination. In eukaryote studies almost all current work is directed towards the question of the control of selective transcription. By analogy with the known control sequences in bacterial genes this has led to the search for common elements in the 5' sequences flanking the gene. But presentations on *Xenopus* 5S RNA genes, sea urchin histone genes, human globin genes and chromatin reconstruction experiments suggest that the control signals need not be in the adjacent 5' sequence and the 5' signals that do exist may not be obvious at the DNA sequence level. Bogenhagen (Carnegie Institution, Baltimore), working with D. Brown, has been asking what sequences of a cloned 5S RNA gene are important for transcription, using the ability of appropriate extracts of *Xenopus* oocytes to obtain precise transcriptional initiation and termination of a cloned 5S RNA gene.

The experiments show that all of the 5' flanking sequence and up to 50 base pairs into the gene may be replaced without substantially affecting initiation of transcription. It follows that the 5' flanking sequences and indeed the first 50 bases of the gene itself do not influence the site of initiation. By progressive deletion from the 3' end of the gene and measuring initiation in the presence of the chain-terminating analogue cordycepin, an intragenic control sequence of 30 base pairs was found. The next step was to insert these 30 base pairs only into a bacterial plasmid and show that the *Xenopus* RNA polymerase III initiated transcription in plasmid DNA 50 base pairs 'upstream' of this sequence in either

orientation. So what is this 'brown box' sequence doing? There are a number of possible explanations but Bogenhagen pointed out that 50 base pairs is 170 Å long and RNA polymerase III is big enough to contact both the intragenic promoter and the 5' initiation point. How many genes have 'brown boxes'? The VA RNAs of adenovirus are transcribed by RNA polymerase III, have a known sequence and are amenable to genetic analysis; these must also be good candidates for intragenic control sequences.

Surrogate genetics

Eukaryote genes coding for proteins are transcribed by RNA polymerase II which has only recently been persuaded to initiate faithfully *in vitro* (Weil *et al.* *Cell*, **18**, 469; 1979). M. Birnstiel (University of Zurich) reported on experiments where genetically manipulated sea urchin histone genes are functionally assessed *in vivo* by microinjection into the nucleus of a *Xenopus* oocyte. The histone genes occur in repeating clusters each containing H4, H2b, H3, H2a and H1 in this order. In the oocyte only H2a and H2b proteins are unequivocally detected when a plasmid containing all the histone genes is injected. Comparison of the noncoding sequences at the 5' and 3' ends of the H2a repeated genes shows conserved regions. The implication is that these regions have a conserved function and the Zurich group is testing whether this is transcriptional control by deleting these sequences and studying RNA transcripts in the microinjection assay. An attractive feature of the system is that H2a may be manipulated while leaving the H2b sequence upstream as an internal control presumably not subject to polar effects. The Zurich group does observe alterations in the amount and size of the H2a transcripts when noncoding 5' sequences are altered *in vitro* although the possibility that RNA processing may be responsible cannot be excluded.

Both the Baltimore and Zurich groups are approaching the question of what is a transcriptional unit using the same logic, of isolation, manipulation and functional assay. R. Flavell (National Institute for Medical Research, London) in contrast, reviewed the descriptive studies of the deletions that give rise to thalassemias in man. He pointed out that deletions of the delta and beta globin gene sequences alter the expression of the fetal globin genes in adult life even though these fetal genes lie more than 10 kilobases to the 5' side of the deleted sequences. There is some tentative evidence that these long range interactions may be a *cis* acting effect. We can conclude that in these three eukaryotic systems our traditional view of what constitutes a gene will be changed. In the case of the *Xenopus*

5S gene there is no doubt that an important new means of exerting genetic control has been described.

Chromatin structures

A. Klug (MRC Laboratory of Molecular Biology, Cambridge) reviewed the impressive evidence accumulating for the solenoid model of nucleosome superstructure from neutron scattering, electron microscopy and, surely definitively, from the X-ray patterns of intact nucleosome crystals. The concern of the Cambridge group has been to describe the higher order structure of chromatin. P. Chambon (University of Strasbourg) on the other hand addressed the question of whether the structural arrangements of DNA in chromatin affect gene expression. The DNase I hypersensitivity of active genes first described by Weintraub now seems to be generally true but as Chambon pointed out it is not known whether this is the cause or consequence of gene activity. Nor is it clear what constitutes a 'domain' of hypersensitivity. In the SV40 minichromosome Varshavsky has shown that a region around the origin of replication is highly susceptible to enzymatic digestion because it is not packaged into nucleosomes. Chambon's group has now shown that this open structure can be obtained on reconstituting SV40 chromatin. The importance of this result is that it shows that the DNA sequence is itself responsible for the 'open' arrangement of nucleosomes around the origin. So in this case at least we can conclude that hypersensitivity to nuclease digestion precedes the expression of the viral genome on infection.

RNA processing

J. Abelson (La Jolla) has made progress in characterising the yeast tRNA splicing activity he has described. The possibility that this 'tRNA splicase' may also be an 'mRNA splicase' was raised by the extracurricular reports of intervening sequences in the yeast actin genes both from Abelson's laboratory and from Gallwitz (Marburg). Irrespective of whether the same enzyme is involved in both tRNA and mRNA splicing, the ease and precision of yeast transformation means that this will be an important organism in the study of the biochemistry and genetics of splicing. H. Robertson (Rockefeller University) reported on work showing that the influenza virion RNA polymerase activity captures the capped 5' end of host mRNA not by splicing but by using the cleaved 5' oligonucleotides as a primer for transcription.

Ron McKay is at the Cold Spring Harbor Laboratory, Long Island, New York.

*The EMBO Symposium on Nucleic Acid — Protein Interactions was held in Heidelberg on 11–15 October, 1979.

Real genetics

Real *Drosophila* genetics has shown that the eye colour mutant white/crimson (W^C) can frequently move its location. W. Gehring (Biozentrum, Basel) in looking for cloned heat shock genes has found a 'copia-like' element (P6F14) that localises to sites predicted by genetic mapping to contain the unstable W^C gene. The so-called 'copia' elements are a family of sequences present at around 30 sites on the chromosomes and which apparently can insert into new sites analogously to the better-known bacterial transposons. The significance of this work is that the dispersed and unstable copia DNA element has picked up a phenotype — in this case the white/crimson. Gehring indicated that in certain stocks the W^C gene may be left in a new location while the unstable element acquires a new phenotype.

M. Ptashne (Harvard University) has used genetic manipulation to mix up the control sequences and proteins of the *lac* genes and the lambda repressor and *cro* gene products. Coupled with DNase protection (footprinting) experiments using the lambda repressor and *cro* gene product he derives an elegant explanation of the role of the three tandem binding sites at the O_R region. There are at least four lessons from this work: (1) 'completely' different proteins can bind to the same DNA sequence; (2) the same protein can be

both a positive and negative transcriptional regulator; (3) cooperative interaction between proteins determines transcriptional control and prophage induction is achieved by *recA* mediated abolition of cooperativity; (4) all temperate phages have three binding sites governing the lytic/lysogenic choice; this arrangement probably prevents frequent mutation to virulence. There is no doubt that the O_R system is the best defined example of the control of transcription. In this case the difference of one base pair in the size of the spacer between the sites is thought to determine the direction of transcription. A. Landy (Brown University) has also used the footprinting technique and genetic engineering to show that the phage λ integration (*int*) gene product interacts with 240 base pairs spanning the core *att* site (at which λ integrates). This promises to explain the mechanism of at least one kind of site-specific recombination. It was left to F. Stahl (University of Oregon, Eugene) in summarising the meeting to make sure that participants obtained a working knowledge of the role of bacterial *chi* sequences in generalised recombination. The implication is that soon bacterial recombination and transcription will be fully explained in terms of known proteins interacting with characterised DNA sequences, leaving the many mysteries of eukaryotes to keep us amused.

provided in the optical by modern observing methods: some new spectrophotometry was thought to be important.

Among extragalactic objects Fe II emission is seen in quasars and the related Seyfert type 1 galaxies. The same multiplets are strong in galactic sources and are identified in the optical region while [Fe II] may be marginally present in one case, IZW1, which has very strong Fe II. There is a great variation in the strength of Fe II relative to other lines or to the continuum. Of the Fe II multiplets, 42 may vary in strength with respect to the others from object to object. One of the most exciting results of the workshop was the convergence of many sets of data finding the same low contrast emission features between 2,000 and 3,000 Å. Both ground-based data on quasars with moderate redshifts by M. Phillips (Cerro Tololo) and H. Netzer (Tel Aviv) and IUE data on low redshift Seyfert galaxies by Snijders and J. Clavel (VILSPA) agreed on the widespread existence of the features illustrated in the Figure. There was however some discussion of the identification of these features: although Fe II may be responsible, other explanations are not yet completely excluded and more work on this point will be done. Generally the intensity of these ultraviolet Fe II features is about equal to that of the optical Fe II lines in spite of factor 100-1000 differences in branching ratios. In addition the IUE observations of Seyfert galaxies with strong optical Fe II may show the presence of some features which could be ultraviolet multiplets in the wavelength range 1600-2000 Å (not previously seen in quasars) and a possibly anomalously strong Si III] to C III] ratio, which would indicate a high density.

Theoretical discussion centred on the Fe II lines in quasars and active galaxies and turned on the energetics. It was agreed that energy can be deposited in the neutral hydrogen, ionised iron region by photoionisation or collisional ionisation. Netzer presented models using a 'mean escape probability' method to handle the radiation transfer which showed that photoionisation by the X-ray continua of active objects could deposit adequate energy in these regions to create the observed Fe II and Mg II lines. Similar calculations by S. Collin-Souffrin and M. Joly (Paris) with a more detailed treatment of the radiation transfer also considered the effects of collisional ionisation. In spite of the difference in fundamental energy source, there was a general similarity in the emergent spectrum which can fit the observed intensity ratios of Mg II to Fe II and of Fe II (ultraviolet) to Fe II (optical). It was also agreed that the excitation mechanism was collisional and not from the resonance fluorescence process. The main argument against the latter is that observations at the Lyman limit in high redshift quasars suggest only a covering

Fe II emission lines in astronomy

from Correspondents

In October the European Space Agency (ESA) sponsored a workshop on the subject of Fe II emission lines in stars and galaxies held at its Villafranca Satellite Tracking Station near Madrid, Spain — also known as VILSPA. It is from this station that ESA carries out its part in the operations of the International Ultraviolet Explorer (IUE) satellite (see *Nature* 275, 5 October; 1979) which can observe the resonance lines of ionised iron in astronomical objects without high redshifts. Before IUE, many objects showing optical emission lines of this ion were known and with the early IUE data came the chance to check theoretical models for the emission from this complex ion by studying the key resonance lines.

Emission lines of ionised iron are widespread in astronomical objects and come from the Sun, all cool stars, some hot stars (in particular those with emission lines, those that are superluminous, the so-called symbiotic stars and binaries), eruptive objects like novae, supernovae, occasional very dense nebulae and extragalactic objects such as quasars and the related Type 1 Seyfert galaxies. In the case of galactic objects these emissions are known to arise in the cool parts of extended envelopes around stars. Typical electron

densities and temperature run in the ranges 10^5 – 10^{11} cm⁻³ and 8,000–10,000 K. Where the densities are less than about 10^8 cm⁻³, forbidden [FeII] emission is expected but J.-P. Swings (Liege) stressed that deducing the density from the ratio of forbidden-to-permitted lines may not always be justified since the lines' profiles may differ sharply between forbidden and permitted lines. Generally the lines that are seen in the optical and ultraviolet emanate from the odd levels around 5 eV, however M. Penston (VILSPA) and M. Snijders (University College London) reported the presence of several strong ultraviolet lines from even 10 eV levels in the slow nova RR Tel and in Nova Cygni 1978 respectively. Both Swings and R. Viotti (Frascati) noted the complex behaviour of the lines, with the relative importance of absorption and emission varying greatly between multiplets and between lines in the same multiplet. M. Friedjung (Paris) reported on the success of curve-of-growth type methods of analysing the Fe II emission spectra of several sources. It was generally agreed that the early investigations of the Fe II emission spectrum in galactic objects had lacked the quantitative dimension now available in the ultraviolet and regularly

Caribou in the Canadian North



are receiving special attention because of apparent physiological adaptations to their especially extreme environment so far north. They accumulate large quantities of fat, they feed heavily on mosses which are avoided elsewhere and conserve energy through reduced activity especially in summer. In spite of the lower plant biomass and annual net primary production in Svalbard compared with areas further south, Svalbard reindeer have a greater population density and faster growth rates. Techniques now in use in studies of reindeer ecology run from remote satellite and aircraft sensing to map range and snowcover as well as to assess range quality, to radionuclide tracer methods used to establish body nitrogen turnover and to estimate energy flow through the lichen-caribou-wolf chain throughout the year. Assuming that the wolves take a constant number of caribou throughout the year, the wolf harvest of caribou was estimated to be 30 ± 5 animals per wolf each year.

Serological techniques for assessing genetic differences and similarities among reindeer and caribou are contributing to the clarification of relationships between the circumpolar racial groups. The Svalbard reindeer are quite unrelated to the reindeer of the Scandinavian peninsula and comparisons with the Peary caribou of the Canadian Arctic have been initiated as an outcome of the symposium.

Man-made obstructions and harassments such as aircraft, pipelines, highways, railroads and powerlines have pronounced effects on the movement, feeding activity and hence energy budgets of reindeer and caribou herds, and the disruption involved in building and servicing oil pipelines for example, with the associated traffic and helicopters, leads to alarm and avoidance reactions especially amongst females accompanied by young. Another unexpected and undesirable side-effect of man's activities has been seen in Sweden and Finland where the fertilisers used in the forestry industry seem to inhibit

the winter foraging for lichens through the snow in these areas, apparently as a result of odours released by the ammonium nitrate and urea fertilisers. □

The neutron half-life and cosmology

from R.J. Tayler

THE recent announcement by Bondarenko *et al.* (*Sov. Phys. JETP Lett.* **38**, 303; 1978) of a new measurement of the neutron half-life giving a value of 10.13 minutes instead of 10.61 minutes, serves as a reminder that this value is of fundamental cosmological importance. A further measurement of the half-life has recently been made at Harwell and a preliminary analysis of the results suggests that the value obtained will also be less than 10.6 minutes (J. Byrne, private communication).

Since the discovery of the microwave background radiation in 1965 there has been a general hope that the hot big bang cosmological theory, originally proposed by Gamow, provides a good first approximation to a description of the evolution of the Universe. One of the key predictions of this theory is the chemical composition of the Universe after the first few minutes. This should be about three parts hydrogen to one part helium by mass, with a very small admixture of deuterium, ^3He and ^7Li . The precise division between hydrogen and helium and the other light isotopes depends on how rapidly the Universe expands in its early stages and on the relative number of protons and neutrons at the time that nuclear reactions converting hydrogen into helium become effective. The simplest version of the big bang theory has one free parameter which can be related to the present mean density of the Universe (ρ_0). The lower the value of ρ_0 , the lower also was the helium production in the early Universe. There is an uncertainty of about two orders of magnitude in ρ_0 and this corresponds to a helium production of between 0.23 and 0.27 by mass.

The rate of expansion of the Universe is increased if there are more stable

elementary particles than is at present believed. In the very early Universe all particles and their antiparticles are expected to be present in equilibrium with the radiation field and stable massless or low mass particles survive through the period of nucleosynthesis and influence the expansion of the Universe. If, as seems very likely, a third neutrino, the tau neutrino, exists, the predicted helium production is increased to the approximate range 0.25 to 0.29. At present there is no unanimity amongst elementary particle physicists about the number of leptons and associated neutrinos, but if there are more than three sets a further increase in helium production is implied.

The half-life of the neutron enters into the problem because it affects all the reactions which convert neutrons into protons. If the half-life is reduced, neutrons are more readily converted into protons and the helium production is reduced. When Gamow first proposed his theory, the half-life was believed to be 14 minutes. By 1965, when the first very serious comparisons between theory and observation were made, the value 11.7 minutes was used and the probable helium production fell in the range 0.25 to 0.29. This was reduced to the 0.23 to 0.27 quoted above, when the accepted value of the half life became 10.6 minutes. A further reduction to 10.1 minutes would give the values 0.22 to 0.26, or 0.24 to 0.28 if the tau neutrino is included.

When the predictions of the theory are compared with all relevant observations, including those of element abundances and those bearing on the value of ρ_0 , it seems just possible that the simplest version of the theory is compatible with observation. Most observed helium abundances are greater than 0.23, although considerably lower values have been observed in the solar wind. It is usually believed that this results from selective loss of elements by the Sun, although Isaak (*Nature*, in the press) has recently claimed that observed solar oscillations and a low neutrino flux demand a low helium content. If we dis-

R.J. Tayler is Professor in the Astronomy Centre, University of Sussex.

Egil Reimers is in the Institute of Zoophysiology, University of Oslo and David R. Klein is with the Alaska Cooperative Wildlife Research Unit, Fairbanks, Alaska.

regard this rather controversial point, the tightest observational constraint on the theory is given by the deuterium abundance, which although very small depends very strongly on the rate of expansion of the Universe.

This agreement between the simplest theory and observation would probably be removed if even a fourth or more species of neutrino were discovered; *inter alia* the predicted helium abundance would be too large. There are more complicated versions of the theory which involve the Universe's being filled with degenerate neutrinos or antineutrinos, which might fit the observations, but these seem rather unattractive. A reduction in the neutron half-life of the amount found by Bondarenko *et al.* would make a small correction in the opposite direction. However, the main significance of the result for cosmologists is to remind us that the predictions of the hot big bang theory are not secure until the neutron half-life is really well known. A definite result that it could not be less than 9.5 minutes (say) would be extremely helpful. □

Amorphous semiconductors

from S.R. Elliott

THE dominant theme at a recent conference on amorphous and liquid semiconductors*, was the hydrogenated amorphous silicon (a-Si:H) system. This is perhaps not too surprising in view of its possibilities as a device material, for example in solar cells (see *News and Views* 275, 93; 1978; 277, 85; 1979). Many of the papers on a-Si:H dealt with aspects of material preparation (for example glow-discharge decomposition of SiH_4 or sputtering in Ar/H_2 atmospheres) or characterisation, such as the measurement of H content by thermal exodiffusion or infrared spectroscopy, and investigations of gap-state density by various field-effect or capacitance-voltage measurements. Few structural studies were reported, although R. Mosseri *et al.* (CNRS, Meudon) compared X-ray diffraction data on a-Si and a-Si:H, and D. Weaire *et al.* (Heriot-Watt University) discussed a hand-built 'ball-and-stick' structural model for a-Si:H containing about 20 at.%H. An entire session was devoted to photovoltaics and solar cell devices, discussed by workers from RCA, Exxon, Dundee and Osaka, and I. Shimizu *et al.* (Tokyo Institute of Technology) described how a-Si:H films could also act successfully as electrophotographic sensors in a manner similar to another amorphous material, selenium, used in copying machines.

A. Madan and S.R. Ovshinsky (Energy

Conversion Devices) reported on fluorinated silicon, a-Si:F:H, (made by the glow-discharge decomposition of SiF_4/H_2 mixtures) which, they claimed, has a lower density of gap states than solely hydrogenated material and which therefore should lend itself to more efficient doping (see also *Nature* 276, 482; 1978). Indeed, for material containing about 5 at. % F, doping with AsH_3 or PH_3 in the gas phase produced material having, at the highest doping levels, conductivities some three orders of magnitude higher than comparable hydrogenated material, although p-type doping with B_2H_6 was found to be comparable in both cases. With a band-gap some 10% larger (rather more suited to absorption of the solar spectrum), a-Si:F:H looks as if it might have considerable device potential. J. Knights (Xerox) presented an interesting paper on growth and morphology of a-Si:H, in which strong evidence from nuclear magnetic resonance, electron microscopy and other techniques was presented for the inhomogeneous incorporation of H. Previously, it had generally been believed that H was uniformly distributed throughout the bulk; Knights, however, showed that high H-containing samples were formed from clusters about 100 Å in diameter, with the H concentrated at the cluster boundaries in the form of SiH_2 configurations. Lower H-containing samples did not show this island morphology, but appeared homogeneous. Plasma chemistry seems to play a major part in the deposition process, as might be expected.

Chalcogenide glasses, the other main area of amorphous semiconductor research, also received much attention. The major breakthrough in this field in the past two or three years has been the realisation that intrinsic structural defects have a major role in determining many electronic properties of these materials. These point defects — dangling bonds — are believed to undergo reconstructions in which electron pairing at these sites occurs. Thus, considering a-Se as an example (in which normally bonded atoms are 2-fold coordinated, forming chains), a simple dangling bond is 1-fold coordinated and contains one electron in the orbital formerly used in bonding. Reconstruction occurs by the transfer of an electron between two such defects: addition of the electron to a centre results in a negatively charged 1-fold coordinated centre: removal of an electron results in an empty orbital capable of forming a dative bond with the full lone-pair orbital on a neighbouring Se chain, thereby forming a positively charged 3-fold coordinated centre. J. Joannopoulos (Massachusetts Institute of Technology) described some of the first self-consistent calculations on such defect configurations in an attempt to obtain total energies. However, it seems that, at least at present, the inability to model the atomic relaxations in a realistic

manner leads to estimates accurate only perhaps to 0.5 eV, unfortunately not precise enough to determine the relative stability of various configurations.

B. Golding (Bell Laboratories) described some fascinating experiments probing low-lying excitations — so-called two-level systems — which seem to exist in chalcogenide glasses. These had been thought to be due to the tunnelling of atomic groups between two nearly equivalent configurations, and they dominate the thermal properties below 1 K. Stimulated emission (in the form of ultrasonic or electromagnetic echo radiation) from these two-level systems can be obtained, and it was shown that such electrical echoes could be altered in magnitude by annealing or by irradiation with light having the energy of the band-gap (or half-band-gap) of the material. The importance of this result lies in the fact that these changes are precisely in the same sense as those observed in photoluminescence or electron spin resonance under the same conditions, properties which are believed to be due to the structural defects mentioned earlier. This is the first direct indication that a connection may exist between two-level systems and structural defects in chalcogenide glasses, an intriguing possibility.

G. Pfister (Xerox) reported on the incorporation of additives (such as transition and alkali metals) in chalcogenide glasses and the effect on properties such as drift mobility and photoluminescence. The discovery that, although the former is dramatically affected, the latter is relatively unaffected by doping means that the simple model for defects in chalcogenide glasses needs to be modified. On a more technological note, a novel method of high resolution image replication using amorphous Ge-Se films was described by K. Chopra *et al.* (Indian Institute of Technology, Delhi). This technique relies on the enormous thickness changes (about 12%) that occur when obliquely deposited films are illuminated with band-gap light; this method has the advantage of being conducted under dry conditions, unlike conventional photolithography.

Finally, the prize for the most unusual and esoteric method of glass production must surely go to a joint Czech and Russian group (L. Stourac *et al.* Czech Academy of Sciences, Prague & Institute of Space Research, Moscow). The experimental environment they used was the weightless condition aboard the Salyut 6 spacecraft, and glass formation and the influence of zero gravity on the structure and optoelectronic properties of Ge-Sb-S glasses were studied. I fear that it will be a little time yet before British researchers have an opportunity to conduct similar experiments! □

S.R. Elliott is University Demonstrator in the Department of Physical Chemistry, University of Cambridge.

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articles

Flux variations of QSO 0957+561 A, B and image splitting by stars near the light path

K. Chang & S. Refsdal

Hamburger Sternwarte, Gojenbergsweg 112, D-2050 Hamburg 80, FRG

If the double QSO 0957+561 A, B is the result of gravitational lens actions by a massive galaxy, stars in its outer parts and close to the light paths may cause significant flux changes in one year. One star can split a QSO image into two to four images with angular separations of $\sim 10^{-5}$ arc s.

RECENT observations¹⁻³ of the double QSO, 0957+561 A, B (redshift $z = 1.405$, angular separation $\alpha = 5.7$ arc s, $\text{mag} \approx 17$) strongly indicate that a large and compact mass (galaxy) lies close to the line of sight between Earth and the QSO. The massive galaxy thereby acts as a gravitational lens. The theory for the case of a point mass (or a compact spherically symmetrical mass distribution) has been presented elsewhere^{4-8,14}. The most convincing proof of a lens effect would be the demonstration of a time delay (of months or years) between flux fluctuations in the two QSOs, which would occur by intrinsic fluctuations in the QSO, as the light travel time is different along the two light paths^{5,6,9,10,14}.

For source diameter < 1 lt yr we show here that flux fluctuations (time scale of months and more) can also be caused by stars, in the outer parts of the deflecting galaxy, moving close to one of the light paths. Such flux fluctuations in the two images would certainly be uncorrelated and show no time delay effect. The absence of a time delay effect should therefore not automatically be taken as disproving the lens effect. The observed light curves would probably show variations due to intrinsic variations in the QSO as well as the influence from the above-mentioned stars. The presence of a time-delayed component in the observed fluctuations would, however, not be difficult to demonstrate.

To simplify the discussion, we consider here the effect of only one star at a time, which is sufficient to demonstrate the importance of stars close to the light path. We shall see that an interesting aspect of the problem is the splitting of each image into a close group of images with angular separation of $\sim 10^{-5}$ arc s. Even one star alone can cause a QSO-image to split into two to four images. Of particular interest is the transition (due to proper motions) from four to two images (or the opposite). Before such a transition occurs, two of the four images are seen to move towards each other, get brighter, and then merge and disappear. The time scale for the disappearance of the two images will roughly be the time for the QSO to move transversely its own diameter D and can be estimated to be about $600D c^{-1}$ in the present case. For D values of < 0.1 lt yr, flux variations of 50% and more can occur and even for D values of ~ 0.5 lt yr a variation of 5% during 30 yr is possible. For such

large D values, however, the different images discussed above will usually merge into one image. From the light curves of the double QSO it should be possible to get information on the size and structure of the QSO as well as on the mass distribution in the outer parts of the deflecting galaxy.

Gravitational lens geometry

The geometry of our problem is indicated in Fig. 1. The heavy solid line represents a light ray coming from Q which is deflected (by the galaxy (G) and the star (S)) when crossing the deflector plane at the point (ξ, η) and which finally crosses the observer's plane at the point (x, y) . The affine parameters, L and λ , are convenient distance parameters for gravitational lens problems at cosmological distances^{11,12}. One might at first think that the Einstein-deflection arising from the nearest star can be neglected since it is only $\sim 10^{-6}$ times the galaxy deflection

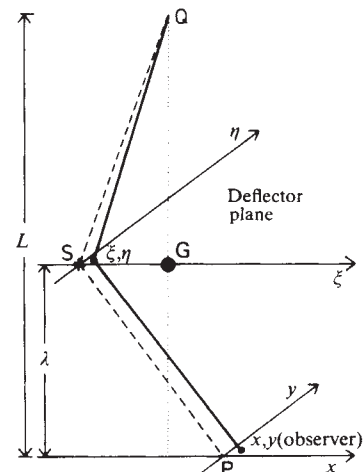


Fig. 1 Light source Q with the affine parameter L (ref. 11) large deflector G (galaxy) with the affine parameter λ and small deflecting mass S (star). The deflector plane (ξ, η) and the observer's plane (x, y) are normal to the line QG. For convenience we chose the origin of the (ξ, η) plane to be at S and the x axis to be parallel to the ξ -axis. The origin P in the (x, y) plane is obtained by considering a light ray QSP for which only the galactic deflection is taken into account. The solid line from Q through the points (ξ, η) and (x, y) represents the path a photon would take when the deflections from G and S are taken into account.

($M_G/M_S \approx 10^{12}$). For the observed fluxes in the observer's plane, however, it is not the values of the deflection angles which count, but rather their derivatives with respect to the deflector plane coordinates, and these derivatives turn out to be of the same order of magnitude.

Basic equations

A suitable form for the transformation of the (ξ, η) plane into the (x, y) plane for the light rays from a point source is the following:

$$x = \xi + \gamma\xi - \xi/(\xi^2 + \eta^2) \quad (1)$$

$$y = \eta - \gamma\eta - \eta/(\xi^2 + \eta^2) \quad (2)$$

The galaxy has here been treated as being symmetrical about QG and with negligible mass outside S. The deflection due to the galaxy is a function of ξ and η and is taken to be the deflection in $\xi = \eta = 0$ plus a term linear in ξ and η . Such a power expansion is a very good approximation since we shall only be interested in light rays which pass S at distances ≤ 1 lt yr. The unit of distance

galactic deflection is taken into account by F'_0 . It is then easily seen from geometrical optics that

$$F'_0/F_0 = |J|^{-1} \equiv \left| \frac{\partial(x, y)}{\partial(\xi, \eta)} \right|^{-1} = |1 - \gamma^2|^{-1} \quad (4)$$

where J is the jacobian determinant of the transformation. The flux ratio of the two observed images A and B of the QSO is $F_A/F_B \approx 4/3$ from the available radio observations². Using the conventional lens effect analysis⁴ (galaxy only) one finds furthermore

$$F_A - F_B = F_0 \quad (5)$$

$$\text{so that} \quad F_A = 4F_0, F_B = 3F_0 \quad (6)$$

We then get an approximate estimate of γ by inserting F_A and F_B instead of F'_0 in equation (4):

$$\gamma_A \approx (1 - F_0/F_A)^{1/2} \approx 0.87 \quad (7)$$

$$\gamma_B \approx (1 + F_0/F_B)^{1/2} \approx 1.15 \quad (8)$$

Equation (4) does in fact allow four solutions of γ for a given flux ratio. It is, however, easy to see that γ must be positive when $M_G > 0$. We also know that the bundle corresponding to the brightest image A has not been through a caustic between source and observer whereas 'bundle B' has been through a caustic. From this it follows that $\gamma_A < 1$ and that $\gamma_B > 1$ and the values given in equations (7) and (8) then follow as the only possible values of γ_A and γ_B .

Critical curves and number of images

An interesting property of equations (1) and (2) is that the inversion of these equations leads to fourth order equations in ξ and η and that one can have two to four solutions for a given x, y . This means that up to four different light rays passing close to S can reach the same point in the observer's plane, so that the observer will see a group of 2-4 images (angular separation $\sim 10^{-5}$ arcs).

To find out how the number of solutions depends on the observer's position (x, y) , the location of the points for which the jacobian of x and y in equations (1) and (2) is zero turns out to be very important:

$$J(\xi, \eta) = \frac{\partial(x, y)}{\partial(\xi, \eta)} = 0 \quad (9)$$

Equation (9) defines the 'critical curves' in the (ξ, η) plane. The transformation of these curves into the observer's plane by equations (1) and (2) then defines the critical curves in the (x, y)

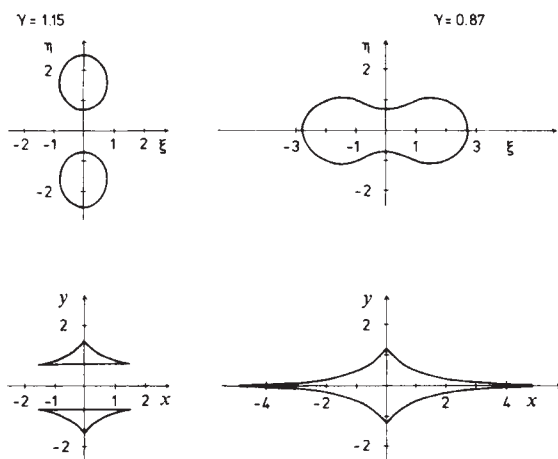


Fig. 2 Critical curves in the deflector plane (ξ, η) and in the observer's plane (x, y) for the cases $\gamma = 0.87$ and $\gamma = 1.15$.

in the (ξ, η) plane has been taken to be the radius r_0 of the luminous ring which would have been seen only if the star had been acting as a lens with the observer directly behind the star. The value of r_0 is^{4,12} (normalised unit),

$$r_0 = \frac{2}{c} \left(GM_S \frac{\lambda(L-\lambda)}{L} \right)^{1/2} \quad (3)$$

For $M_S \approx 1 M_\odot$ and for cosmological distances the value of r_0 is of the order of 0.1 lt yr. Finally, the normalised unit in the (x, y) plane is $r_0 L/(L-\lambda)$. An interesting feature of equations (1) and (2) is that there is only one free parameter γ and that the γ -terms arise from the galactic deflection.

If we neglect the effects of the deflecting star, we can drop the third term on the right-hand side in equations (1) and (2):

$$x = \xi + \gamma\xi \quad (1a)$$

$$y = \eta - \gamma\eta \quad (2a)$$

We denote the flux which would have been observed without any lens effect by F_0 and the corresponding flux if only the

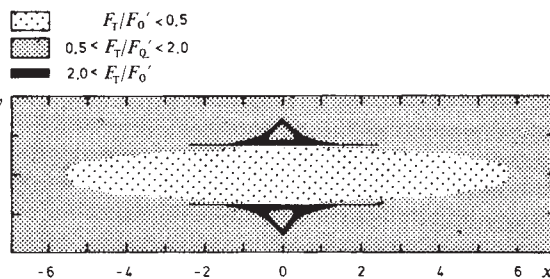


Fig. 3 The observer plane, for the case $\gamma = 1.15$, divided into a high flux region, an intermediate flux region and a low flux region. F'_0 is the flux which would have been observed, if only the galactic deflection is taken into account (the star is neglected).

plane. The critical curves in the (ξ, η) plane are found to be Cassini ovals and they are plotted for the cases $\gamma = 0.87$ and $\gamma = 1.15$ in Fig. 2 together with the corresponding critical curves in the (x, y) plane. These two cases also demonstrate the two types of critical curves one can obtain. For $\gamma < 1$ one closed curve is always found in each plane; for $\gamma > 1$, however, there are always two closed curves.

By using topological arguments one can show that the number of images of a point source seen by an observer can only change when he crosses a critical line, and that the number of images then changes by two. This is also in perfect agreement with our numerical experience; we always find that the observer sees two images when he is outside the critical curve(s) and four solutions when he is inside one of the critical curves.

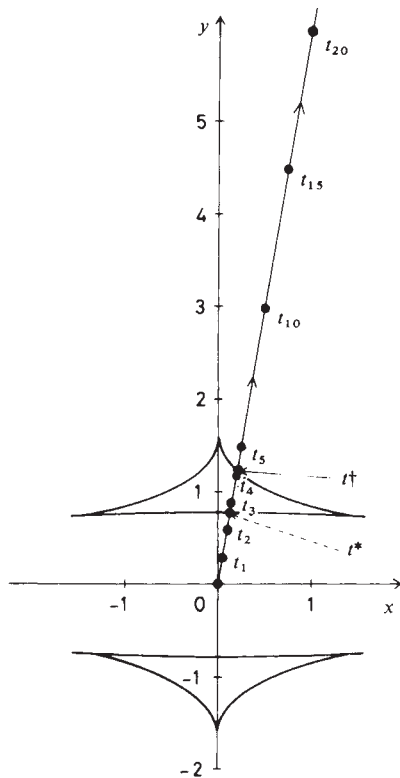


Fig. 4 Motion of the observer in the (x, y) plane ($\gamma = 1.15$) for the case discussed as an example in the present investigation. The direction of the observer's motion is indicated by an arrow.

Flux values

Since the images discussed above will have very small angular separations ($\approx 10^{-5}$ arc s) we shall mainly be interested in the total observed flux F_T from all the images

$$F_T = \sum_{i=1}^N F_i, \quad N \leq 4 \quad (10)$$

Here F_i is the observed flux from image i . In analogy with equation (4), but now using equations (1) and (2) instead of equations (1a) and (2a) one gets for a point source

$$F_i = J^{-1}(\xi_i, \eta_i) F_0 \quad (11)$$

As the light rays from B pass closer to the galaxy (that is pass through a denser star field) than those from A, the chance of getting a large disturbing effect from a star should be greater for B than for A. We therefore concentrate on B ($\gamma = 1.15$).

Figure 3 shows the main features of the function $F_T(x, y)/F_0$. We see that a high flux region (star causes more than +100%) is

found on and close to the critical curves, whereas a low flux region (star causes a reduction of 50% or more) is found in an extended region around the x -axis. The high F_T -values close to the critical curve ($J = 0$) were, of course, to be expected, since a vanishing of the jacobian for at least one of the images means an infinite flux (within the point source and geometrical optics approximation). For sources which are small but finite, one can show that the maximum value of F_T which occurs when an observer passes a critical line is proportional to $D^{-1/2}$, where D is the diameter of the source. Note that the same type of dependence was found for the spikes discussed by Benson and Cooke¹³. The large low flux region obtained in the present calculation does not occur in the case of a single point mass lens ($\gamma = 0$) discussed extensively elsewhere^{4,7}. Observers within 0.75 distance units from the origin would even find that the flux is reduced by more than 90%. This is in striking contrast to the single point mass case as then a high flux region is found close to the origin. The region with 100% increase or more reaches for instance 0.55 distance units out from the origin in the (x, y) plane.

Motion in the observer's plane

Rough estimates of the transverse velocities (normal to QG) of Q, S (ref. 2) and the observer lead to an observer's velocity relative to P ($x = 0, y = 0$) of about $1,000 \text{ km s}^{-1}$. The unit of distance in the observer's plane which was chosen to be $r_0 L / (L - \lambda)$ depends on the redshifts, the Hubble parameter H_0 and slightly on the cosmological model. For galaxy redshifts

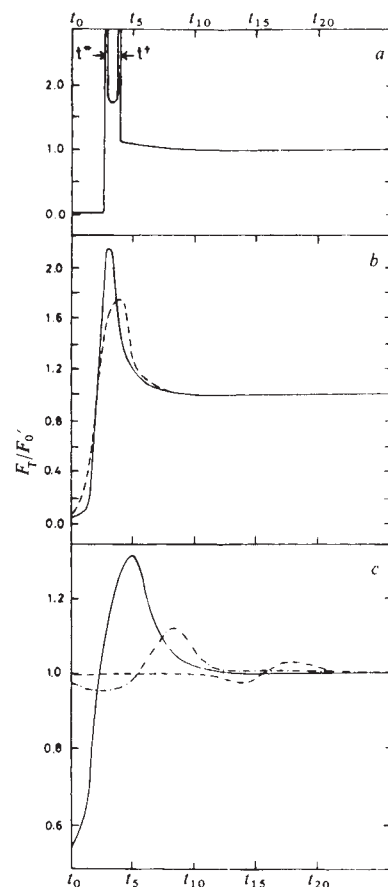


Fig. 5 Light curves with different values of D' . The real diameter of the source object Q is given by $D' r_0 L / \lambda$. a, Point source; b, $D' = 0.5$, --- $D' = 1.0$; c, — $D' = 2.0$, - - - $D' = 4.0$, . . . $D' = 10.0$.

$z_G \approx 0.5$ and $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$ we find approximately

$$r_0 \cdot L / (L - \lambda) = 0.1 \text{ lt yr} \quad (12)$$

a value which we shall now adopt. A motion amounting to one distance unit in the observer's plane (relative motion between observer and P) will then take with velocity $1,000 \text{ km s}^{-1}$ about

$$\Delta t = 30 \text{ yr} \quad (13)$$

An example of a motion in the observer's plane is shown in Fig. 4. Starting at time $t = t_0$ at the origin, the motion at constant velocity is followed through times $t_1 = t_0 + 0.3 \Delta t$, $t_2 = t_0 + 0.6 \Delta t$ till $t_{20} = t_0 + 6 \Delta t$.

Point source

The change in the total flux F_T is demonstrated in Fig. 5a for the case of a point source. It is seen that $F_T(t)/F'_0$ starts out as being very small and stays < 0.1 until the critical curve is reached at time $t = t^*$. Then an abrupt rise to very high values occurs (infinite by geometrical optics approximation). Very close to a critical curve (on the inside) the value of F_T/F'_0 is proportional to $d^{-1/2}$, the constant of proportionality being ~ 1 if the distance d to the critical line is expressed in normalised units. Therefore one has a minimum in F_T before the next crossing of the critical curve at $t = t^\dagger$, then one again finds very high F_T values. An abrupt reduction to values $F_T/F'_0 \approx 1.2$ outside the critical curve follows. The positions of the different images in the (ξ, η) plane (on the sky) is demonstrated in Fig. 6. Starting with two images on the ξ axis one can see that a 'new' very bright image appears at $t = t^*$ and that this image immediately splits into two images. One of these images then merges with one of the original images at $t = t^\dagger$ and the joint image disappears.

Extended sources

We now consider circular sources without limb darkening and with various diameters D . The source centre is in Q and the observer motion is the same as for the point mass case. The time dependence of F_T/F'_0 is shown in Fig. 5b and c for various values of D' . D' is the diameter of the source transformed into the observer's plane (just as the point source is transformed into point P) in normalised units. It is then easily seen that the diameter of the source is

$$D = D' r_0 \lambda L^{-1} \approx 0.05 D' \text{ lt yr} \quad (14)$$

We have used $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $M = 1 M_\odot$. For the most reasonable cosmological models ($0 < q_0 < 1$) and redshifts z_G ($0.3 < z_G < 1$) equation (14) is accurate to $\pm 40\%$.

As expected we find that the amplitudes of the flux variations get smaller and the time scales larger when D' increases. By the largest D' value shown in Fig. 5 ($D' = 10$) we still see, however, that a 5% increase in $\sim 30 \text{ yr}$ is possible. If we consider flux variations of $< 1\%$ as insignificant we find that significant flux variations can only occur for $D' < D'_{\text{crit}} = 20$. From equation (14) we see that this corresponds to

$$D < D_{\text{crit}} = 1 \text{ lt yr} \quad (15)$$

Stars as deflectors can therefore only give significant flux changes for D smaller than about 1 lt yr.

As for the position and appearance of the extended images, we note that a large increase in the flux of an image is always connected with a large deformation, and that the separate images which appear of a point source often smear together for large D' values.

For small D' values the rapid flux changes when the observer crosses a critical line is of utmost interest. We have found that the maximum value of F_T/F'_0 when crossing a critical line is usually about ($D' \leq 0.1$)

$$F_T/F'_0 \approx 2/\sqrt{D'} \quad (16)$$

The time scale for the disappearance or appearance of the bright images is

$$\delta t = D' \Delta t \quad (17)$$

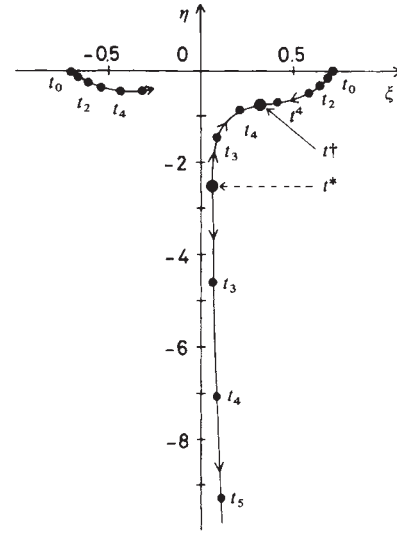


Fig. 6 Motion of the images in the deflector plane.

The very rapid X-ray fluctuations (we think intrinsic) of some quasars (time scale some hours), which have been reported from the Einstein satellite, do indicate that the D -value of the X-ray source is less than $5 \times 10^{-4} \text{ lt yr}$; that is $D' < 0.01$. Large changes in F_T caused by the lens effect could then occur within some months.

Probability considerations

We have seen that significant changes in the total flux F_T can occur for sources less than about 1 lt yr, if a star happens to be close enough to the light path. The probability that such flux changes will actually occur depends on the number density of stars in the deflector plane. For simplicity we consider all stars to have one solar mass and to be lying in the deflector plane. One can then easily show that the average number density $\langle n \rangle$ of stars out to a distance GS from G is $\langle n \rangle = \pi^{-1}$ per normalised area (r_0^2). We have here made use of equation (3) for the whole galaxy, that is, with M_G instead of M_S . We can then write the number density of stars close to the light bundle as

$$n = \frac{1}{\pi} \frac{n}{\langle n \rangle} \quad (18)$$

The number density of projection points P in the observer's plane (per normalised area $r_0^2 L^2 / (L - \lambda)^2$) is

$$n' = (F'_0/F_0) \cdot n = \frac{3}{\pi} \frac{n}{\langle n \rangle} \approx \frac{n}{\langle n \rangle} \quad (19)$$

as the lens effect of the galaxy reduces the projected area in the observer's plane with a factor F'_0/F_0 . Noting that the low flux region in the observer's plane ($F_T < 0.5 F'_0$) has an area of 12 it is clear that the probability of large flux changes is significant even for $n/\langle n \rangle$ values as small as 0.01. We consider more likely values of $n/\langle n \rangle$ to be ~ 0.1 . In that case the disturbance from several stars at a time would usually have to be considered.

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The heat flow field in mainland UK

S. W. Richardson & E. R. Oxburgh*

Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford, UK

Belts of relatively high flux correspond to zones of crust enriched in radioactive heat production to ~15 km depth. The enrichments were introduced into the crust by granitic magmas.

MOST of the ~80 measurements of heat flow in the mainland UK¹⁻⁵ have been made in vertical or near vertical holes between 200 and 500 m deep from which both thermal conductivity and equilibrium thermal gradient information have been obtained. Data available up to October 1977 are summarised elsewhere¹⁻⁵, since when 26 more values have been obtained.

Although the distribution of observations is much less uniform than desirable and is determined largely by the availability of suitable boreholes drilled by other bodies, it is clear that heat flow varies in a more or less systematic manner by as much as a factor of three, between different parts of the country. Figure 1 shows the distribution of observations, and contours for 45, 60 and 75 mW m⁻². The contours are drawn by hand on the basis of the observations shown, without reference to any additional constraints. There are clearly different, equally possible placings for the lines shown, but from most of these emerge the broad regional patterns. A zone of relatively high heat flow extends northwestwards across central and northern England and may continue into southern Scotland (probably terminating in the Scottish Midland Valley). A second, less well defined, belt runs from Cornwall in extreme south-west England eastwards along the south coast of the country. A relatively low heat flow area occupies the western English Midlands and much of central and north Wales. The few values of north of the Scottish Midland Valley are all rather low.

Although a much better distribution of observations is required to substantiate this pattern, the observation density makes this one of the most thoroughly studied areas of its size in the world, and patterns of heat flow variation on this scale have not previously been described.

The average heat flow for the whole country is close to 60 mW m⁻² (see Fig. 1), the lowest value recorded being 25 mW m⁻² and the highest 130 mW m⁻². The precise value for the mean depends on the method used to derive it, but clearly the country is on average not notably different from other stable continental areas⁶⁻⁸. The average heat flow is, for example, close to the European average⁹, 64 mW m⁻², and in general the values found in the UK are between the low regional flux of Scandinavia and the higher flux of central and southern Europe.

Correlation of heat flow pattern with other features

Perhaps the most striking feature of Fig. 1 is the relatively poor correlation between the heat flow pattern and other regional geophysical features. Although not presented here, maps of bouguer gravity and aeromagnetic anomalies were prepared as overlays to the heat flow map; the former shows no obvious regional correlation at all, while there is a weak correlation between the trends of magnetic anomalies and the trends of the two belts of higher heat flow values.

There is similarly little correlation with the surface geology. A weakly folded cover of Mesozoic rocks conceals the detail of the folded Palaeozoic and Precambrian basement over much of

England¹⁰⁻¹². In south-west England the zone of higher heat flow corresponds approximately to the trend of the Hercynian Fold Belt characterised by folded slates cut by granitic intrusives; the heat flow contours appear to close eastwards as the belt is buried by the Mesozoic cover.

The northern England zone of higher heat flow apparently cuts across the sedimentary and tectonic features of the Upper Palaeozoic basement, and the Lower Palaeozoic is very poorly known except from isolated outcrops and boreholes. The zone does, however, correspond to the subsurface trend of a hypothetical belt of folded Lower Palaeozoic rocks recently proposed by Evans¹³.

The ill-defined low heat flow area contained between the two higher zones, corresponds in its eastern part to a region believed to be largely underlain by Precambrian rocks but extends westwards into north and central Wales where folded Lower Palaeozoic rocks occur at the surface.

Compilations of continental heat flow show that there is a broad dependence of values on the age of the last thermal event to have affected the crust^{7,14-17}. The majority of the values shown in Fig. 1 come from crust which is in this sense 400–250 Myr in age and exhibit no correlation with the age. The variation of heat flow reported here must thus represent the considerable scatter found in the broad-scale compilations.

Origin of the variation in surface heat flow

The fact that the mean heat flow for the country as a whole is not exceptional, together with the scale width of the variation in surface heat flow, suggest that the origin of the pattern is not to be found in some unusual condition in the mantle but rather reflects some crustal condition or process¹⁸⁻²⁰.

Even very slow and low-volume flows of water in the crust may transfer amounts of heat by convection which are of the same order as the vertical conductive flux^{2,21}. Such flows must, however, be driven either by a topographic head, or by initial horizontal temperature differences. Viewing the regional heat flow pattern as a whole there is no obvious correlation with topography or with the known pattern of near surface groundwater movements²². On the other hand, there are almost certainly areas where water movements do control the heat flow on a local scale (for example, the limestone area of south Derbyshire¹).

The thermal conductivity of rocks commonly varies by a factor of two between different lithologies^{2,3}. A crust characterised by adjacent belts of high conductivity and low conductivity rocks would be expected to refract any uniform heat flux supplied to it from below, so that the higher conductivity regions carried a higher heat flow, at any rate near their margins^{24,25}. We have considered the possibility that the high heat flow belts of Fig. 1 are formed in this way and simple two-dimensional analytical and numerical modelling has been carried out. We find that steep sided zones with twice the conductivity of their surroundings, and extending to a depth of half, or even the full thickness of the crust, show strong refractive edge effects which perturb the surface heat flow by ~±20%. This would be just sufficient to explain the amplitude of the observed variation but the sharp depressions and elevations of heat flow at the junction of the conductivity domains do not match the observed broad areas of low flux and intervening belts with distinctly higher flux throughout. Despite these general considerations there is little doubt that on a local scale refraction of heat flow is important²⁶.

In regions which have not experienced recent igneous or metamorphic activity a significant fraction of the surface heat

* Present address: Department of Mineralogy and Petrology, University of Cambridge, Downing Place, Cambridge, UK.

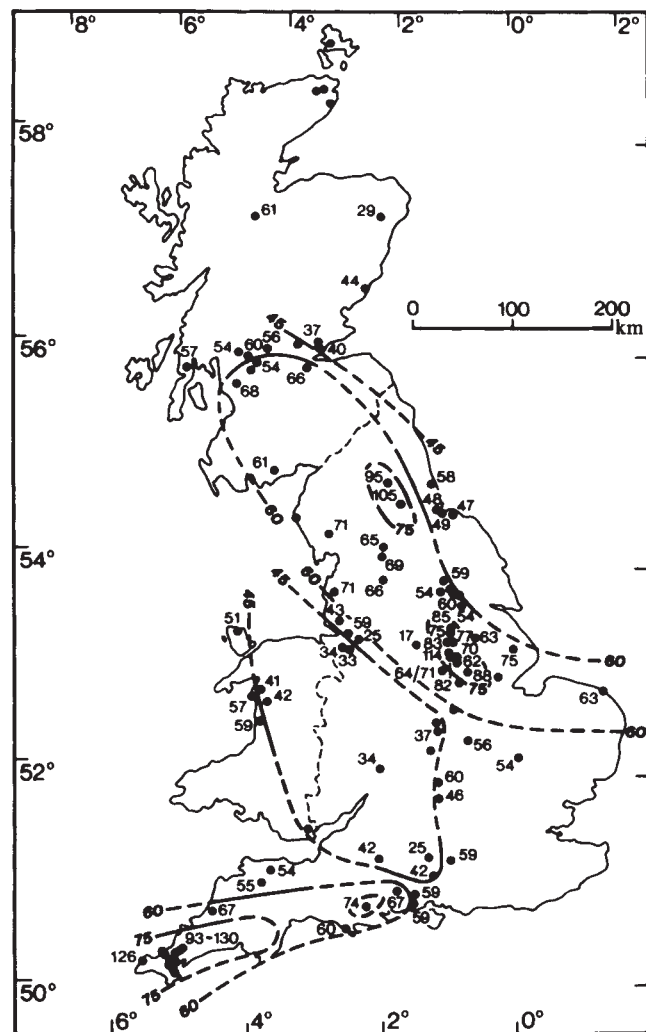


Fig. 1 Heat flow sites with the values contoured at 45, 60 and 75 mW m^{-2} . Although uncertainty attends the placing of the contours, particularly across the Scottish Southern Uplands and in Wales, the two belts of higher values stand distinctly above background.

flow of continents is derived from heat producing isotopes which are strongly concentrated in the continental crust with respect to mantle abundances. At equilibrium, the surface heat flow may be expressed as:

$$q_0 = q_m + \int_0^m A \, dz \quad (1)$$

where m refers to the depth (z) above which enrichment is both significant and variable; q and A refer to heat flow and heat productivity respectively. Studies of several continental regions²⁷⁻²⁹ suggest that the integral in equation (1) can be solved simply as $A_0 D$ on the empirical grounds that q_0 and the surface heat production are linearly correlated in regions where high grade gneissic, or granitic intrusive rocks outcrop. The parameter D in these terrains has a characteristic value of 8.0 ± 1.5 km and q_m accounts for about half the average heat flow observed²⁹. We have previously reported heat flow and productivity measurements for southern Britain¹ which showed good correlation where either granitic or low grade cleaved metasediment or metavolcanic rock outcrop, or are encountered as basement in boreholes. These and further data for Britain, including southern Scotland, are plotted in Fig. 2 which support the correlation ($R = 0.96$); D has a value of 16.6 km. This relationship implies that the granitic and weakly metamorphic basement of Britain, whether outcropping at the surface or buried beneath a platform of younger sediment, is enriched in

heat production to depths of ~ 15 km and that the degree of enrichment is geographically variable and causes the heat flow variation.

Although, in principle, such an explanation could account for the large scale features of Fig. 1, it does not explain how such variation in heat production could have come about. We have argued elsewhere¹ that much of the basement to these depths comprises slates and other low grade metamorphic rocks of sedimentary origin. Thus perhaps the simplest explanation of the heat flow pattern is that it maps out areas beneath which sediments of differing bulk heat production are preserved. Such differences could reflect either the provenance or the depositional environment of the sediments. However, the contours of heat flow, in northern Britain (Fig. 1) at least, cut across apparently discrete depositional zones such as the Midland Valley and Southern Uplands of Scotland³⁰.

All the highest values of heat flow in Britain have been measured in holes penetrating granites with high heat production and several bodies of this kind occur at the surface in the southern high heat flow belt, and shallowly in the subsurface of the northern belt. The deep structure and possible connections between these bodies has been the subject of research for some time³¹⁻³³ and a possible explanation for the high heat flow zones is that they correspond to zones of high heat-production intrusions presumably associated with old orogenic belts. Note that all the granites are too old for there to be detectable effects associated with their original cooling after employment.

The systematic variations of chemical composition of acidic and intermediate igneous suites normal to the trend of such belts have been extensively studied³⁴ and have generally been related to special conditions associated with partial fusion of a descending lithospheric slab³⁴⁻³⁶. Thus the chemical anomalies implied by the high heat flow zones could be regarded as features introduced into the crust at the time of granitic magmatism. In the present cases the southern high heat flow belt would be a Hercynian feature and the northern zone a Caledonian feature following the trend suggested by Evans¹¹. If it could be demonstrated that the northern belt was indeed continuous from the Lake District across the Southern Uplands to the Midland Valley of Scotland, its formation would clearly postdate the closing of the Southern Uplands crustal suture³⁰.

The correlation in Fig. 2 implies some degree of communication between heat production in granitic rocks and in slates, because the same scale length for heat production distribution applies to either rock type. It is envisaged that such communication could be either physicochemical, or purely physical, or most likely some combination of the two.

A chemical communication could be set up by the interchange of fluid between granites and their country rocks. The cooling of granitic bodies may induce groundwater circulations^{37,38} which are able to scavenge incompatible elements, such as those responsible for heat production, from large volumes of country rock and reprecipitate them within the granites and their immediate surroundings. Figure 2 would imply that the scale length of the convective process, and hence the fluid interchange, was ~ 15 km. It is, however, unlikely that redistribution of heat production could, by itself, give rise to the observed heat flow belts. The pattern expected from such a process alone would be one in which zones of elevated flux were flanked by exactly compensating zones of depressed flux resulting from hydrothermal scavenging. In view of the observed generally low heat flow outside the belts the main effect of the granitic rocks is probably to provide initial enrichment to the belts.

A purely physical relationship between heat production distribution within granites and slates can be envisaged in a two-layer crust; the lower layer would consist of relatively dense rocks of uniform heat production within which granites are too buoyant to reside for the period of their crystallisation. The upper layer would comprise the slates themselves and their intrusive granitic stocks. On this interpretation (a similar one to that proposed by Smithson and Decker³⁸) heat production in each rock type would be uniform at all depths within the upper

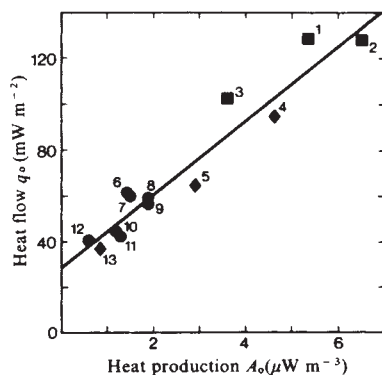


Fig. 2 Surface heat flow plotted against radioactive heat production in local granitic or low-grade metasedimentary and metavolcanic basement. Regression line: $q = 16.6A + 27$. Correlation coefficient $R = 0.96$. ■, Hercynian granites of south-west England. ♦, Lower Palaeozoic granites. ●, Cleaved phyllitic basement. Data for points 1, 2, 4, 5, 7, 8, 9, 11, 12 and 13 are summarised in ref. 1. Point 3 is Longdowns³. Points 6 (Langstrothdale) and 10 (Montrose) (unpublished data). The slope and intercept of the correlation would be substantially unchanged were granitic rocks alone to be considered.

layer; the slope of the correlation in Fig. 2 is thus directly interpreted as the mean thickness of the upper layer.

Conclusions

We conclude that the main features of the heat flow field in mainland UK reflect lateral variations in the concentration of heat producing elements within the upper half of the crust. These in turn arise from the distribution of high heat-production granitic bodies which introduced heat production anomalies into an upper crust which was initially predominantly slaty in character. Although now cool the granites may, at the time of their intrusion, have initiated large scale convective circulations of water within the crust, which redistributed trace elements in sedimentary and igneous rocks alike. Enrichments in heat production might be expected to sustain some low-volume circulation over long periods of time. On a local scale the heat flow field is modified both by present day water flows and by heat flow refractions.

The relatively long scale length of the heat production distribution implies that near-surface temperature gradients in, for example, the Cornish granites should extend virtually unmodified to depths of several kilometres. Note that the same

processes which redistribute heat-producing elements might also be expected to concentrate other elements in ore deposits. There is a weak correlation between the high heat flow areas and exposed zones of epigenetic mineralisation. This correlation may extend into areas where older rocks are hidden beneath a cover of Mesozoic and younger sediments.

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Organisation and sequence studies of the 17-piece chicken conalbumin gene

M. Cochet, F. Gannon, R. Hen, L. Maroteaux, F. Perrin & P. Chambon*

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de L'INSERM, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France

The conalbumin gene has been cloned and shown to consist of at least 17 exons approximately 60–200 base pairs long. The DNA sequence upstream from the region coding for the 5' end of the mRNA shows similarities with sequences present in homologous positions in other genes. High and low frequency repetitive sequences are found both upstream from the conalbumin gene and within one intron.

CONALBUMIN (or ovotransferrin) is one of the major egg white proteins synthesised by the tubular gland cells of the magnum portion of hen oviduct. Its expression is controlled at the transcriptional level by oestrogens and progesterone, as are the other egg white proteins (see ref. 1 and refs therein). Nevertheless there are some differences in the patterns of expression of these genes after hormone administration. For instance, oestradiol administration results in a very rapid increase of conalbumin mRNA (con-mRNA), whereas the appearance of ovalbumin mRNA is somewhat delayed (see ref. 2 and refs therein). Comparison of the DNA sequences flanking these genes might

* To whom correspondence should be addressed.

therefore provide a means of testing the idea that steroid hormones act (at least in part) through interactions of steroid-receptor complexes with the genome (see refs 3 and 4 and refs therein). In addition, the study of the conalbumin gene and its flanking sequences provides an interesting insight to the problem of tissue-specific expression of a unique gene¹.

Oviduct conalbumin and liver transferrin are the same protein, differing only in the nature of their carbohydrate moiety^{5,6}. However, the modulation of the expression of the conalbumin-transferrin gene is very different in the two tissues. The expression of the oviduct gene is much more sensitive to oestradiol administration than is that in the liver, despite the presence of oestradiol receptors in chicken liver⁷. In addition, iron levels appear to be involved in regulating the expression of the gene in the liver, but not in the oviduct (G. S. McKnight, D. Lee and R. Palmiter, personal communication).

In previous studies we have constructed a recombinant plasmid containing a doubled-stranded cDNA (dscDNA) copy of con-mRNA (pBR322-con1, (ref. 8)) and have used it to investigate the structure of the conalbumin gene¹. Restriction enzyme mapping of the genomic DNA revealed that the conalbumin gene is split and is contained in three *EcoRI* fragments, 5'-*Eco* 'b'-*Eco* 'c'-*Eco* 'a'-3', about 4.0, 2.5 and 11.0 kilobase pairs long, respectively (see Fig. 1A(b); ref. 1). The *EcoRI* fragments 'b' and 'c' and about 1,500 base pairs at the 5' extremity of fragment 'a' were cloned in the λ phage recombinant λ C4-con1 and shown to contain the sequences coding for the first 940 nucleotides of the 2,400 nucleotide-long con-mRNA¹.

We now report the molecular cloning of the conalbumin *EcoRI* fragment 'a', which allows us to describe the organisation of the complete conalbumin gene. The DNA base sequence

within and upstream from the region coding for the 5' end of con-mRNA has also been obtained and the existence of repeated sequences within the conalbumin gene and upstream from its 5' end is reported.

Cloning of conalbumin *EcoRI* fragment 'a'

Since previous attempts to isolate the complete *EcoRI* fragment 'a' from libraries representing the whole chicken genomic DNA had been unsuccessful¹, we decided to start from a DNA fraction enriched in this fragment. A recombinant, λ WconA1, hybridising to a probe specific for fragment 'a', was isolated as outlined in the legend to Fig. 2. Digestion of this cloned DNA with *EcoRI* revealed the presence of a DNA fragment of a similar size to that of the genomic conalbumin *EcoRI* fragment 'a' (Fig. 2, lanes 1 and 2). Comparison (Fig. 2) of the restriction enzyme patterns obtained from the cloned and the partially purified cellular fragment 'a' digested with *XbaI* (lanes 3 and 4), *BamHI* (lanes 5 and 6), *BglI* (lanes 8 and 9) and *BglII* (lanes 10 and 11), confirmed that the cloned fragment is indeed the conalbumin *EcoRI* fragment 'a'.

Combined with unpublished and previously reported mapping¹, the present restriction enzyme digestions allow the construction of the conalbumin gene restriction enzyme map shown in Fig. 1A(b). In addition, we have used (results not shown) the con-dscDNA probes, *EcoA* and *EcoB* (Fig. 1B(a)), *Eco-BglI*, *Eco-BglII* and *Eco-BamI* to localise, on the genomic DNA map, the *EcoRI*, *BglI*, *BglII* and *BamHI* sites of con-dscDNA (Fig. 1A(a), (b)). The *Bam2* site of the dscDNA (Fig. 1A(a)) was not found in the genomic clone. This discrepancy is accounted for by the existence of allelic variants for this *BamHI* site (our unpublished results and D. Lee, G. S. McKnight and

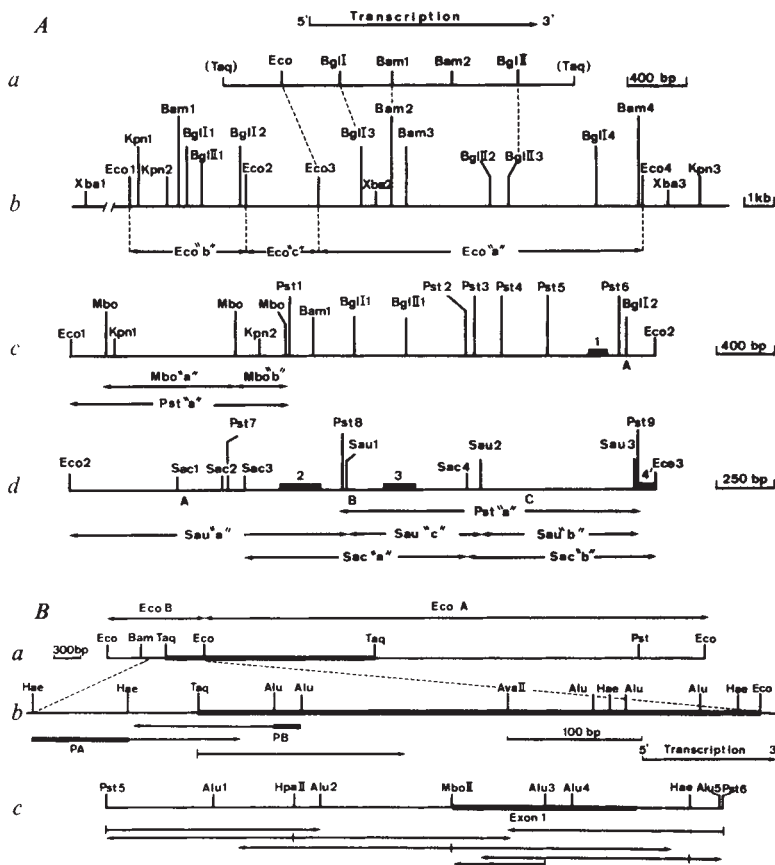


Fig. 1 A, Organisation of the sequences coding for conalbumin mRNA in chicken erythrocyte DNA and in genomic DNA clones. a, Partial restriction endonuclease map of the dscDNA copy of con-mRNA and scale in base pairs (bp) (taken from ref. 8). b, Restriction endonuclease map of the erythrocyte DNA region containing the conalbumin gene and scale in kilobase pairs (kb). This map extends from that previously published¹ to include the *BamHI*, *BglI* and *BglII* sites. These sites were mapped (our unpublished results) using the previously cloned *EcoRI* fragment 'b' (ref. 1) and the presently cloned *EcoRI* fragment 'a' (see text and Fig. 2). The sequences coding for con-mRNA are contained in *EcoRI* fragments 'a', 'b' and 'c'. The dotted lines between a and b indicate the correspondence between the restriction enzyme sites found in the dscDNA and the genomic clones (see text). c, Restriction endonuclease map of the cloned *EcoRI* fragment 'b' and scale in bp. For each restriction enzyme all of the existing sites are shown except for *MboII* where only the relevant sites are shown (see text and Fig. 6). The number 1 corresponds to exon 1 and A represents the 5' moiety of intron A¹. d, Restriction endonuclease map of the cloned *EcoRI* fragment 'c' and scale in bp. A, B, C, 2, 3, and 4' are the 3' moiety of intron A, intron B, intron C, exon 2, exon 3 and the 5' moiety of exon 4, respectively. **B, Strategy for DNA sequencing at the 5' end of conalbumin double-stranded cDNA and around exon 1 in the cloned genomic DNA.** a, Partial restriction enzyme map of pBR322-con1⁸ linearised with *EcoRI* and scale (in bp). The heavy line corresponds to the inserted con-dscDNA. The two *TaqI* sites were generated during the insertion of the con-dscDNA in the *SalI* site of pBR322⁸. *EcoA* and *EcoB* correspond to the fragments which are generated by *EcoRI* digestion of pBR322-con1^{1,8}. b, DNA sequencing at the 5' end to con-dscDNA and scale (in bp). The pBR322-con1 fragment *EcoB* (see a) was mapped by digestion with various restriction enzymes either singly or in combination and the restriction enzyme map of a selected region is represented. Due to the small size of the fragments resulting from *AluI* digestions, it was necessary to label them at their 5' end with [γ -³²P]-labelled ATP and T4 polynucleotide kinase in order to reveal them by autoradiography. Primers A and B (PA and PB) were prepared by acrylamide gel electrophoresis of *AluI* or *HaeIII* digests of purified fragment *EcoB*¹. They were used to sequence around the *Taq* site by using the chain termination method of Sanger *et al.*¹⁰ and the extent of the sequenced regions are shown by the arrows. The third arrow indicates the extent of the region sequenced from the *Taq* site using the method of Maxam and Gilbert¹¹ (*Taq-Eco* fragment end-labelled with [γ -³²P]-labelled ATP and cut with *AvaII*). c, DNA sequencing within and around exon 1 of the genomic DNA. The *PstI*-*Pst6* fragment of *EcoRI* fragment 'b' (1Ac) was subcloned in pBR322, isolated on sucrose gradient after *PstI* digestion and mapped with various restriction enzymes as outlined under b. DNA sequencing was carried out using the Maxam and Gilbert method¹¹. The arrows indicate the direction and the extent of the sequence determinations from the 5' end-labelled restriction enzyme sites. When a given region was not sequenced on the two strands, it was sequenced at least twice on the same strand to ensure the accuracy of the sequence determination. The abbreviations used for restriction enzymes are for *EcoRI*, *BamHI*, *TaqI*, *PstI*, *HaeIII* and *AluI*. Sequencing gels and additional details about the strategy are available upon request. Scale as in Fig. 1B(b).

R. Palmiter, personal communication) similar to those which were previously described for the ovalbumin gene^{9,12-15}.

13 introns are present in the conalbumin *EcoRI* fragment 'a'

Seven conalbumin exons (1-7) separated by six introns (A-F) have previously been localised in *EcoRI* fragments 'b' and 'c' and in the previously cloned 1,500 base pair long 5' segment of fragment 'a' (see ref. 1, and Figs 1A(b) and 4f and g). The cloning of fragment 'a' allows one to determine whether the ~2,000 nucleotides of con-mRNA which are not encoded in *EcoRI* fragments 'b' and 'c' are completely encoded in fragment 'a'.

Electron microscopy of hybrid molecules formed between con-mRNA and the cloned genomic *EcoRI* fragment 'a' reveals that con-mRNA hybridises to *EcoRI* fragment 'a' over $2,048 \pm 184$ nucleotides in 14 exonic RNA-DNA hybrid regions (4'-17), separated by 13 intronic single-stranded DNA loops (D-P) (Figs 3A and 4b). As expected from our previous results¹ the length measurements show that the first 1,500 base pairs at the 5' extremity of fragment 'a' contain the previously identified introns D, E and F, the 3' moiety of exon 4 and exons 5 and 6. These observations definitely prove that the conalbumin *EcoRI* fragments 'c' and 'a' are contiguous. The organisation and the length of the various exonic and intronic sequences present in *EcoRI* fragment 'a' are given in Fig. 4b, g.

There is excellent agreement between the restriction enzyme map and the electron microscopic map. From the comparison of these two maps (Fig. 4a, b) one can predict that the restriction sites *Bam*2, *Bam*3 and *Bgl*II2 should be located in exon 9, intron 1 and intron N, respectively. These predictions were verified by hybridising con-mRNA with specific restriction enzyme segments (Fig. 3). For instance, hybridisation with the *Eco*3-*Bam*2 and the *Bam*3-*Bam*4 fragments (Fig. 4a) allows one to localise *Bam*2 and *Bam*3 sites in exon 9 and in intron 1, respectively (Figs 3B and 4c, d). Hybridisation of con-mRNA with *Eco*3-*Bgl*II2 fragment (Fig. 4a) indicates that site *Bgl*II2 is located in intron N (Figs 3C, 4e). Also, the *Bgl*II3 and *Bgl*II3 exonic sites (see above and Fig. 1A(a), (b)) appear to be located in exons 7 and 16, respectively (Fig. 4a, b).

The conalbumin gene is in 17 pieces

From our present and previous results¹, it appears that the conalbumin gene consists of at least 17 exons separated by 16 introns (we cannot exclude the existence of additional introns too small to be detected by electron microscopy). This striking structure is visualised in Fig. 3D in the form of a hybrid molecule between con-mRNA and the linked cloned conalbumin *EcoRI* fragments 'b', 'c' and 'a'. Seventeen exonic RNA-DNA hybrid regions (1-17) separated by 16 intronic, single-stranded DNA loops are visible (Figs 3D, 4f, g). The length of the entire DNA-RNA hybrid region is $2,508 \pm 164$ base pairs, in very good agreement with our previous estimate of con-mRNA length of about 2,400 nucleotides⁸. Since there is no clearly visible tail at the 3' end of the hybrid region (see also Fig. 3A, B) despite the existence of a poly(A) sequence attached at the 3' end of con-mRNA, it is very likely that exon 17 contains the DNA sequence coding for the 3' end of con-mRNA. Exon 1 contains the DNA sequence coding for the 5' end of the mRNA (see below). It appears therefore that con-mRNA is entirely encoded in the 10.3 kb genomic region which is between the 5' end of exon 1 and the 3' end of exon 17. This conclusion is supported by the observation (M. LeMeur and A. Krust, personal communication) that the longest con-mRNA precursor molecule which can be found in oviduct RNA is 9,900-10,100 nucleotides long.

The 5' end of conalbumin mRNA is encoded in exon 1

Since the sequence of con-mRNA was totally unknown and we previously showed that the con-dscDNA plasmid pBR322-con1

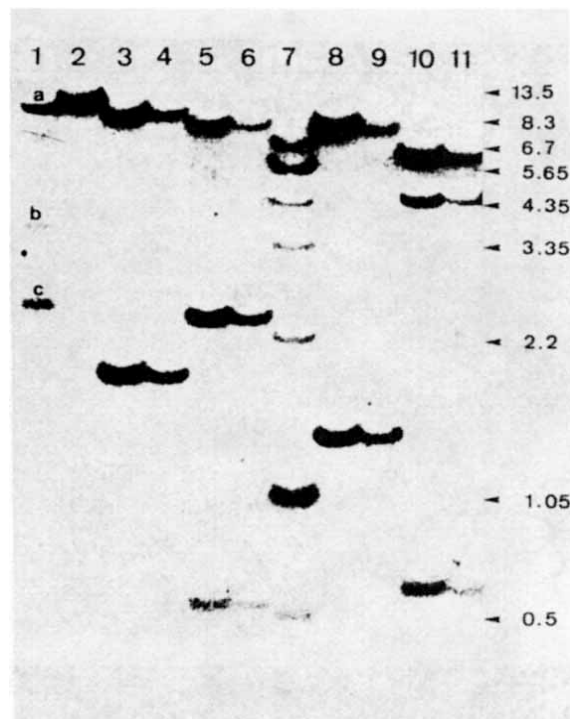
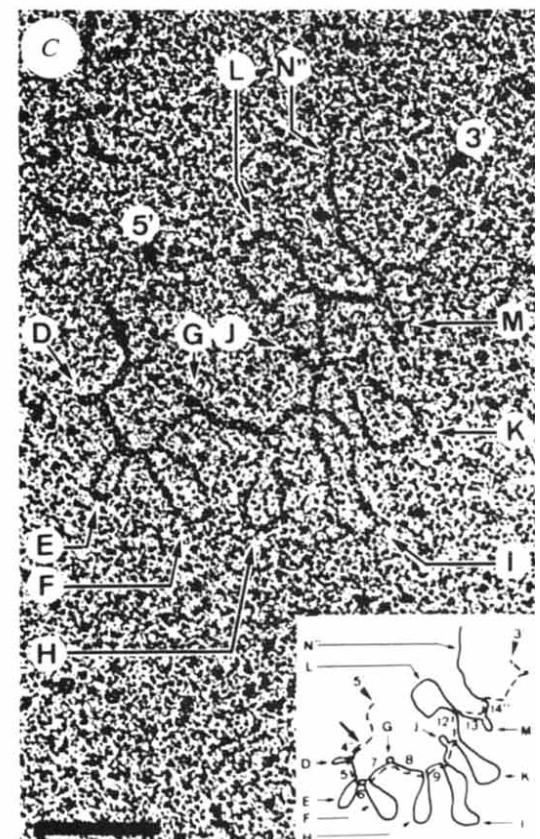
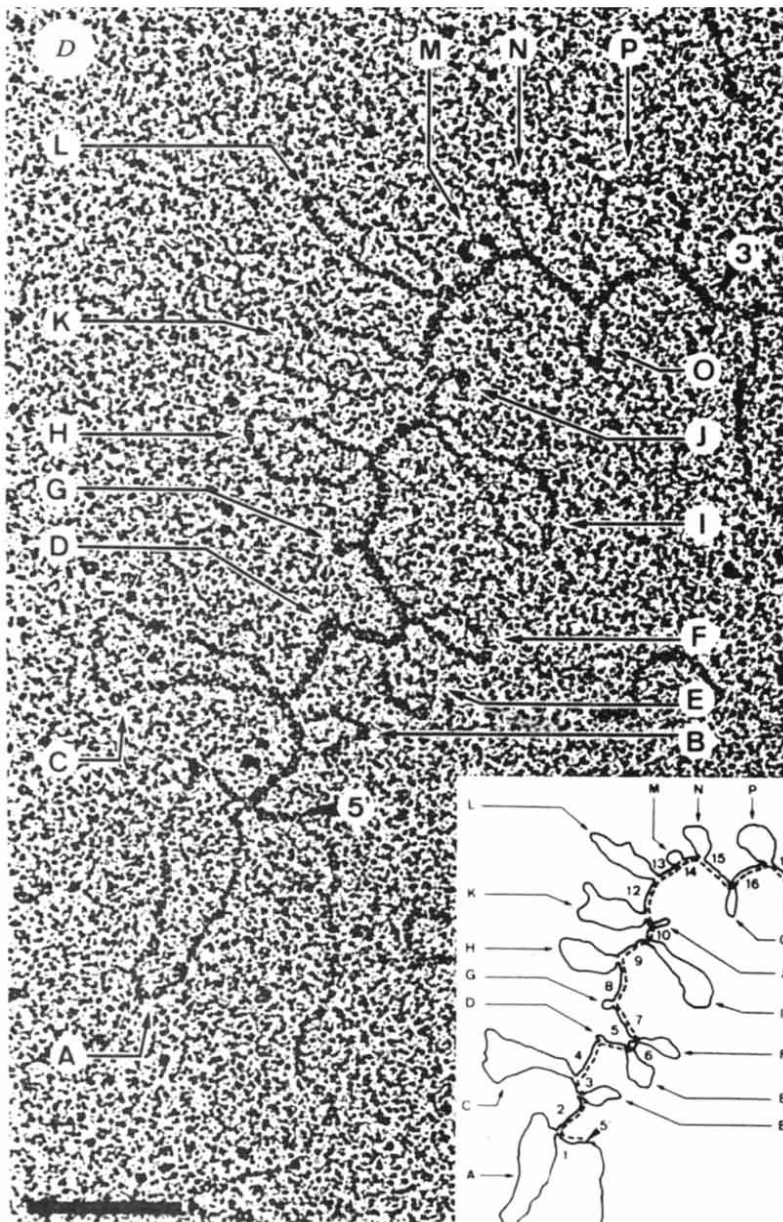
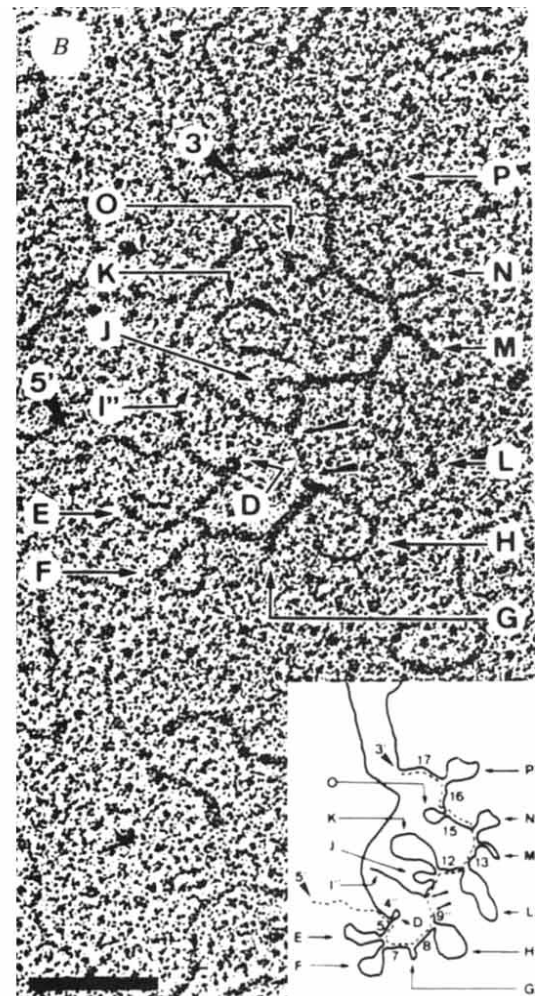
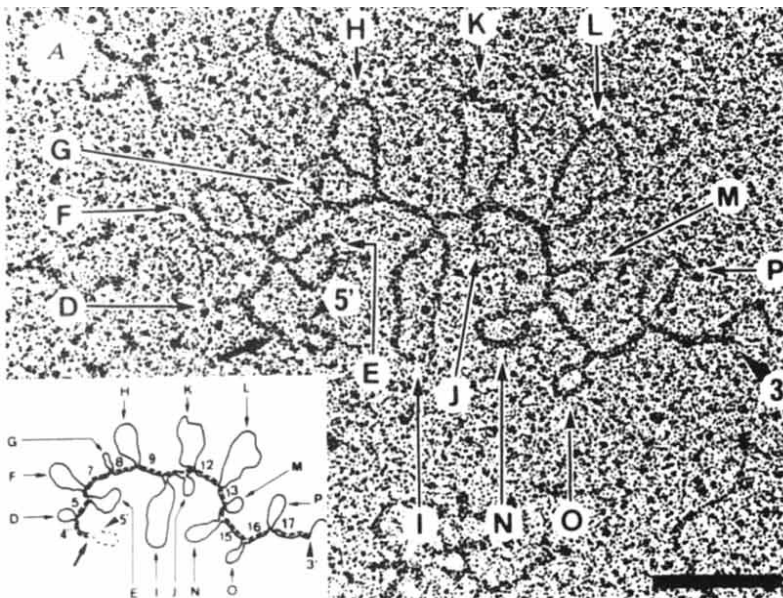


Fig. 2 Comparison of restriction enzyme fragments obtained from the cloned and partially purified cellular *EcoRI* fragments 'a'. The cloned *EcoRI* fragment 'a' was obtained as follows: 0.25 µg of an *EcoRI* digest of erythrocyte DNA partially purified by two cycles of RPC-5 chromatography⁹ and sized by sucrose gradient centrifugation, was ligated with 0.6 µg of *Ag*tWES.ΔC right and left arms and subsequently packaged and cloned as previously described by Garapin *et al.*¹⁶ using the conalbumin dscDNA probe specific for the sequences coded for by *EcoRI* fragment 'a' (*Hha* B probe¹). Out of 10,000 recombinants which were screened, one (λWconA1) hybridised to the probe and was amplified. About 2 µg of RPC-5 partially purified erythrocyte DNA (see above) (lanes 3, 5, 8, 10) and 0.3 ng of an *EcoRI* digest of λWconA1 DNA (lanes 4, 6, 9, 11) were digested with *Xba*I (lanes 3 and 4), *Bam*HI (lanes 5, 6), *Bgl*II (lanes 8 and 9) and *Bgl*III (lanes 10 and 11), electrophoresed on a 1% agarose slab gel, transferred onto nitrocellulose filters, hybridised with ³²P-labelled con-dscDNA probe pBR322-con1⁸ and revealed by autoradiography as previously described^{1,17}. Lanes 1 and 2 contained an *EcoRI* digest of total erythrocyte DNA (15 µg) and λWconA1 DNA (0.3 ng), respectively. ('a', 'b' and 'c' indicate the position of the three genomic *EcoRI* fragments.) Lane 7: hybridising size markers derived from pBR322-con1 plasmid. The size of the hybridising fragments (in kilobases) are: Lane 1: (a) 11, (b) 4, (c) 2.5; lane 2: 11; lanes 3 and 4: 9.1 and 1.9; lanes 5 and 6: 8, 2.45 and 0.55; lane 7: 6.7, 5.65, 4.35, 3.35, 2.2, 1.05 and 0.5; lanes 8 and 9: 7.9 and 1.45; lanes 10 and 11: 5.8, 4.6 and 0.6.

[Fig. 1B(a)] should contain most of the sequences corresponding to the 5' end of the mRNA⁸, we first sequenced the 5' end of con-dscDNA. The sequence shown in Fig. 5A was obtained using the Maxam and Gilbert¹¹ and Sanger *et al.*¹⁰ methods as outlined in the legend to Fig. 1B(b). The first possible AUG initiation codon is found 57 base pairs downstream from the 5' end of the dscDNA. This AUG codon is the initiation codon for conalbumin, since it is followed (with one exception, see below) by the sequence coding for the 19 amino acids present at the N-terminal of pre-conalbumin (protein leader sequence, or signal peptide⁶) and the first amino acid (alanine)^{6,18} of mature conalbumin. There is one difference at position -2 of the leader sequence of amino acids between our version derived from DNA sequencing and that of Thibodeau *et al.*⁶ obtained by amino acid sequencing. This discrepancy does not seem to be related to DNA or protein sequencing inaccuracy (R. Palmiter, personal communication) and may reflect the existence of two allelic variants of the conalbumin gene similar to those mentioned above for *Bam*HI site.

Since it was very likely that the DNA sequence corresponding to the 5' end of the dscDNA would be found in the genomic exon 1, we sequenced the *Pst*5-*Pst*6 fragment of *Eco* 'b' which



contains exon 1 [Fig. 1A(c) and B(c)]. The sequence is presented in Fig. 5A. As expected, we found a region where the genomic and the dscDNA sequences were identical. This region, which contains the *Alu3* and 4 sites, codes for the first 14 amino acids of the protein leader sequence. The limit between exon 1 and intron A is given by comparing the cellular and the dscDNA sequence (dashed line). The exonic-intronic junction sequence resembles the model sequence¹⁹ which was derived by comparing several exon-5'-intron junction sequences and, once again, the dinucleotide GT is present at the 5' end of an intron, in agreement with the 'GT-AG rule'¹⁹.

Although the above results confirmed that exon 1 contains sequences coding for the 5' region of con-mRNA, sequencing the 5' end of the mRNA was necessary to determine the location of the 5' extremity for exon 1 and whether it contains the sequence coding for the 5' end of the mRNA. The sequence of the 5' end of con-mRNA was obtained by using the genomic *Alu3-Alu4* fragment [see Fig. 1B(c)] as a primer and the modified chain termination method of Sanger *et al.*^{10,20} in order to sequence reverse transcripts of mRNA (see legend to Fig. 5B). With the exception of the first nucleotide, the sequence of the first 60 nucleotides of the mRNA was obtained and is presented in Fig. 5A. A sequencing gel corresponding to the first 36 nucleotides is shown in Fig. 5B. The first nucleotide of the mRNA cannot be determined with this method and the band which is present in all four lanes (lanes 1-4 of Fig. 5B) above the first U corresponds presumably to the first nucleotide of the mRNA as indicated by the position of an [*Alu3-Alu4*]-primed reverse transcript of the mRNA synthesised in the absence of dideoxynucleoside triphosphates (Fig. 5B, lane 5). The first nucleotide of the mRNA should therefore be an A by comparison with the cellular DNA sequences shown in Fig. 5A. The nature of the first nucleotide of the mRNA was confirmed as follows: an *Alu2-Alu3* fragment end-labelled with [γ -³²P]-labelled ATP and polynucleotide kinase, was hybridised to con-mRNA under conditions which favour DNA-RNA hybrid formation²¹, digested with *S*₁ nuclease, and electrophoresed on an acrylamide gel under denaturing conditions in parallel with the chemically cleaved products for DNA sequencing according to Maxam and Gilbert¹¹ of the same region. The migration of the major *S*₁-protected fragment corresponds to the T, which in the coding strand of the genomic sequence, is complementary to the A numbered 1 in the non-coding strand (Fig. 5A) (R. Hen, unpublished result). The first nucleotide of con-mRNA is therefore probably an A. However, direct RNA sequencing will be required to definitely establish the 5' end sequence of con-mRNA. From the present study we conclude that exon 1 contains the sequence coding for the 5' end of con-mRNA and that there is a 76 nucleotide-long untranslated region at the 5' end of con-mRNA. Therefore con-mRNA, in contrast to ovalbumin and some viral mRNAs (see refs 19 and 24 and refs therein), but like other eukaryotic mRNAs, do not have an untranslated leader sequence encoded in a separate exon at its 5' end.

Presence of repetitive sequences within and around the conalbumin gene

None of the conalbumin mRNA-coding sequences is repeated in the chicken genome¹. Studies on the ovalbumin^{9,16} and globin²² genes have shown that the intronic sequences of these genes are also unique. In contrast, when a restriction enzyme digest of total chicken DNA is blotted onto a nitrocellulose filter and hybridised with either conalbumin *EcoRI* fragment 'b' or 'c' labelled by nick-translation, a very fast and complete darkening of the autoradiographic film is observed (not shown). This indicates the presence of highly repetitive sequences in both the *EcoRI* fragments.

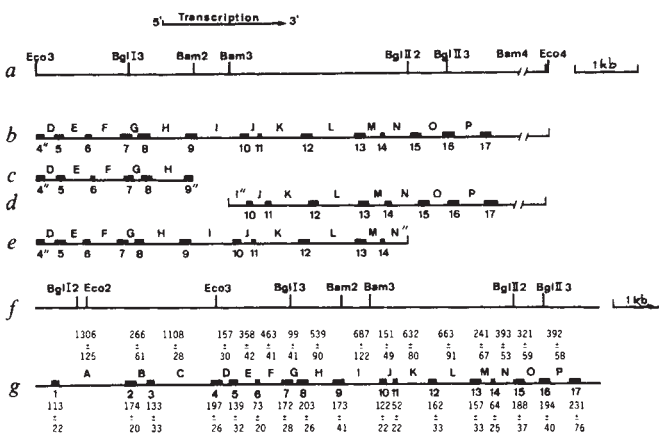


Fig. 4 Schematic representation of the hybrid molecules between con-mRNA and cloned genomic DNA fragments as measured by electron microscopy. **a**, Restriction enzyme map of conalbumin *EcoRI* fragment 'a' (see Fig. 1A(b)) and scale in kb for **a-e**. **b**, Localisation of exonic and intronic sequences contained in cloned *EcoRI* fragment 'a' (see Fig. 3a). Heavy lines (4'' and 5-17) refer to DNA-RNA hybrid (3' moiety of exon 4 and exons 5-17, respectively) regions. **c**, Localisation of exonic and intronic sequences within the *Eco3-Bam2* and *Bam3-Bam4* fragments (see Fig. 3b). Notation as under **b**, except for 9'' (the 5' moiety of exon 9) and I'' (the 3' moiety of intron I). The lengths of 4'', 9'' and I'' are 140 ± 22, 87 ± 44 and 296 ± 60 base pairs, respectively. The lengths of the other exons and introns are as shown in **g** within the experimental error. **e**, Localisation of exonic and intronic sequences within the *Eco3-Bgl12* fragment (see Fig. 3c). Notation as under **b** except for N'' (the 5' moiety of intron N). The lengths of 4'' and N'' (in base pairs) are 113 ± 30 and 358 ± 94, respectively. The lengths of other exons and introns are as under **g** within experimental error. **f**, Restriction enzyme map of the conalbumin gene region (see Fig. 1A(b)) and scale in kb for **f** and **g**. **g**, Localisation of the exons and introns in the conalbumin gene as established from the present data and those previously reported for the cloned conalbumin *EcoRI* fragments 'b' and 'c' and the 5' moiety of *EcoRI* fragment 'a'¹. Letters A-P and numbers 1-17 (heavy lines) correspond to the conalbumin gene introns and exons, respectively. The length of the exons 5-17 and of introns D-P are taken from the measurements made on 25 hybrid molecules similar to that shown on Fig. 3a, whereas the length of exons 1-4 and introns A-C are taken from Perrin *et al.*¹.

Fig. 3 Electron micrographs of hybrids between conalbumin mRNA and cloned genomic DNAs. The hybrid molecules were prepared as described previously¹. In the line drawings, the dashed line represents the RNA and the solid line the DNA. Capital letters refer to intronic sequences, and numbers to the exonic RNA-DNA hybrid sequences (for the sake of clarity not all of the exons are numbered in the line drawings). The 5' and 3' ends of the con-mRNA molecules are indicated with arrows. **a**, Hybrids between con-mRNA and heat-denatured cloned *EcoRI* fragment 'a' purified from λ WconA1 DNA. The RNA-DNA hybrids (2,048 ± 184 base pairs) are interrupted by 13 single-stranded DNA loops (D-P) (see line drawings and Fig. 4b). The length of the 3' moiety of exon 4 (4'') and those of exons 5-17 together with the length of introns D-P are given in Fig. 4b, g and their legends. The unnumbered arrow points to the 5' end of the RNA-DNA hybrid region which corresponds to site *Eco3* (see Fig. 4a, b). **b**, Hybrids between con-mRNA and heat-denatured *Eco3-Bam2* and *Bam3-Bam4* fragments of the cloned *EcoRI* fragment 'a'. The *Eco3-Bam2* fragment (Fig. 4a) was subcloned in pBR322 and the recombinant plasmid was linearised with *Bam*HI before heat-denaturation. The *Bam3-Bam4* fragment (Fig. 4a) was subcloned in pBR322 and excised with *Bam*HI before heat-denaturation. The fragment *Eco3-Bam2* contains introns D-H, the 3' moiety of exon 4 (4''), exons 5-8 and the 5' moiety of exon 9 (9'') (see line drawing and Fig. 4c), whereas fragment *Bam3-Bam4* contains the 3' moiety of intron I (I''), introns J-P and exons 10-17 (see line drawing and Fig. 4d). The two unnumbered arrows delimit the regions of the mRNA encoded by the 3' moiety of exon 9 which is contained in the *Bam2-Bam3* fragment (Fig. 4a, b) which was not present in this hybridisation. Length measurements are given in the legend to Fig. 4c, d. **c**, Hybrids between con-mRNA and heat-denatured *Eco3-Bgl12* fragment of cloned *EcoRI* fragment 'a'. The *Eco3-Bgl12* fragment (Fig. 4a) was prepared by digestion of the purified cloned *EcoRI* fragment 'a'. The hybrid regions contain the 3' moiety of exon 4 (4''), exons 5-14 and introns D-M and the 5' moiety of intron N (N''). (See line drawing and Fig. 4e for length measurements.) The unnumbered arrow points to the 5' end of the RNA-DNA region which corresponds to the *Eco3* site. **d**, Hybrid between con-mRNA and the cloned conalbumin gene. Hybrids were formed between con-mRNA and the heat-denatured complete conalbumin gene plasmid pBcon2 which was constructed from a modified pBR322 vector (containing a *Kpn*I site) by inserting the *Kpn2-Eco3* fragment (Fig. 1A(b)) and the *EcoRI* fragment 'a' in the genomic order (M. Cochet, unpublished results). *EcoRI* fragment 'a' was purified from λ WconA1, whereas the *Kpn2-Eco3* fragment was obtained from λ WconP5¹. The hybrid region (2,508 ± 164 base pairs, 12 hybrid molecules were measured) contains all of the conalbumin exons 1-17 separated by the intronic loops A-P (see line drawing). In all cases the bars represent 0.1 μ m.

These sequences were localised by restriction enzyme mapping using, as nick-translated probes, the two fragments and the total chicken DNA. Fragment *Eco* 'b' hybridises with both *Eco* 'c' (Fig. 6A, lane 1) and cellular DNA (Fig. 6A, lane 4) probes. The region which hybridises with both probes is localised in the *Pst* 'a' fragment [Fig. 6A, lanes 2 and 5, and Fig. 1A(c)]. However, the *Eco* 'c' probe hybridises only with the *Mbo* 'b' fragment [Figs 6A, lane 3; 1A(c)], whereas the cellular DNA probe hybridises strongly with fragment *Mbo* 'a' and only slightly to fragment *Mbo* 'b' [Fig. 6A, lane 6 and Fig. 1A(c)]. From these results we conclude that in a region located 2,000–3,200 base pairs upstream from the 5' end of the conalbumin gene there is a highly repetitive sequence localised in fragment *Mbo* 'a' and a sequence situated in fragment *Mbo* 'b', which is repeated in *Eco* 'c', but not repeated very often in the rest of the genome. (We use the term highly repetitive, because these sequences must be repeated several hundreds of times in the genome for the total chicken DNA to be used as an efficient hybridisation probe in blotting experiments.)

Similarly, fragment *Eco* 'c' hybridises with *Eco* 'b' (Fig. 6B, lane 1) and cellular DNA (Fig. 6B, lane 4) probes. The region which hybridises with probes is localised in the *Pst* 'a' fragment of fragment *Eco* 'c' [Fig. 6B lanes 3 and 6, and Fig. 1A(d)], but the *Eco* 'b' probe hybridises only with the fragment *Sac* 'a' [Fig. 6B, lane 2, and Fig. 1A(d)], whereas the cellular DNA probe hybridises essentially with fragment *Sac* 'b' [Fig. 6B, lane 5, and Fig. 1A(d)]; on longer exposure exposure a faint hybridisation to fragment *Sac* 'a' was observed). From these results we conclude that the 3' moiety of intron C [fragment *Sac* 'b', Fig.

1A(d)] contains a highly repetitive sequence, whereas the sequence which is similar to that present in the *Mbo* 'b' fragment of *Eco* 'b' is located in the 5' moiety of intron C or in the 3' moiety of intron B and is not repeated very often in the rest of the genome. Also, since the highly repetitive sequence present in *EcoRI* fragment 'b' is not revealed by *Eco* 'c' probe and conversely, our results demonstrate that the highly repetitive sequences contained in the conalbumin *EcoRI* fragments 'b' and 'c' belong to two different families of repetitive sequences.

It is interesting to compare our results with those recently reported by Eden and Hendrick²³, who found, by reassociation kinetic studies, that the repetitive chicken DNA can be divided into two classes based on repetition frequency: about one-third of the sequences are repeated 15-fold and two-thirds are repeated 1,500-fold. Also, they found that 40% of the chicken genome is arranged in an alternating pattern of single copy and repetitive sequences, with single copy regions of about 4,500 nucleotides in length, whereas almost one-half of the DNA consists of very long single copy regions, uninterrupted by repeated sequences for distance at least 17,500 base pairs. The two highly repeated sequences probably belong to the class of sequences which are repeated about 1,500-fold in the genome. It is striking that they are separated by 4,500 base pairs of single copy DNA as predicted from the reassociation kinetic studies. In contrast, their length is much shorter than that predicted by Eden and Hendrick²³ (at most 600 to 900 base pairs, instead of 2,000 base pairs). In contrast, there is no highly repeated sequence in the 10-kilobase pair-long conalbumin *EcoRI* fragment 'a' (L.M., unpublished result). In this respect, it is

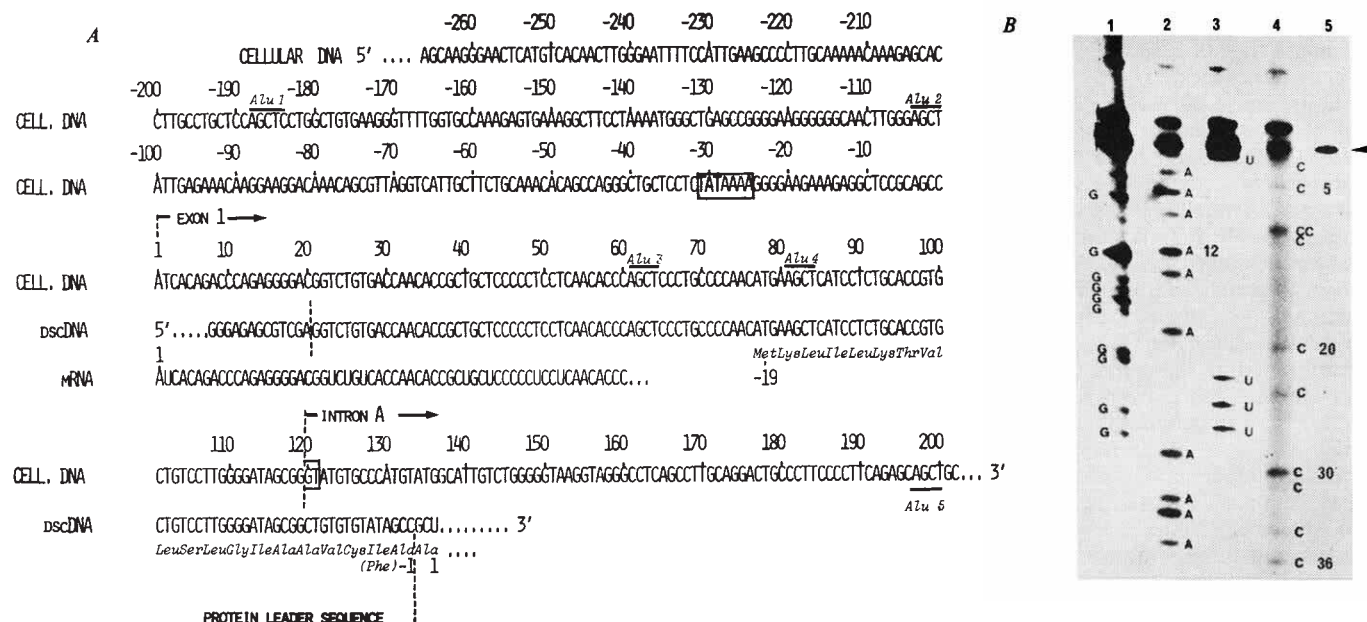


Fig. 5 A, Comparison of nucleotide sequences at the 5' ends of conalbumin double-stranded cDNA (dscDNA) and conalbumin mRNA (mRNA) with the corresponding sequences from genomic DNA (cell DNA). The nucleotide sequences at the 5' end of con-dscDNA and the genomic sequences within and around exon 1 were established as outlined in Fig. 1B. The first 60 nucleotides of con-mRNA were determined as described below and exemplified in B for the first 36 nucleotides. The DNA sequences (non-coding strand) were aligned with the mRNA sequence. The positive numbers refer to position of the nucleotides downstream from the 5' end of mRNA, whereas negative numbers are given upstream from nucleotide number 1. The limit of exon 1 and of the 5' end of intron A is shown in the genomic DNA sequences by a vertical dashed line. The dinucleotide GT¹⁹ at the 5' end of intron 1 is boxed. The positions of the *Alu* sites (*Alu*1 to *Alu*5) shown in Fig. 1B(c) are indicated. The possible promoter AT-rich region (see text and Fig. 7B) is boxed. The limit separating the pBR322 sequence (TCGA corresponding to the *Taq*I site, see Fig. 1B(b) from the 5' end of the dscDNA is indicated by a dashed line. The amino acid sequence of the protein leader sequence of pre-conalbumin⁶ and the first amino acid of conalbumin are shown under the con-dscDNA sequence. A dashed line separates the protein leader sequence from the first amino acid (labelled 1) of the mature protein. The only amino acid difference (at position -2) between the sequence derived from the dscDNA and that obtained by amino acid sequencing⁶ is indicated. B, Autoradiogram of sequencing gel showing the sequence at the 5' end of con-mRNA. The sequence was obtained by the chain termination method of Sanger *et al.*¹⁰ as modified by Akusjärvi and Petterson²⁰ in order to sequence reverse transcripts of mRNA. An *Alu*I digest of 0.3 µg of the genomic *Pst*5-*Pst*6 fragment (see Fig. 1B(c)) was denatured and hybridised with 60 µg of total hen RNA (purified on oligo dT-cellulose) as described in ref. 20, but for 1 h at 37 °C. The chain elongation (using [α -³²P]-labelled TTP) and the chase step were carried out at 37 °C as in ref. 20, but reverse transcriptase was added again before the chase step which was prolonged for 16 hours. Lanes 1-4 correspond to the reaction mixtures containing the dideoxynucleotides ddCTP, ddTTP, ddATP and ddGTP, respectively (the bases indicated in the figure correspond to those of the mRNA and are numbered from its 5' end). The position corresponding to the first nucleotide of the mRNA was determined as follows: an *Alu*3-*Alu*4 fragment labelled at its 5' ends with [γ -³²P]ATP and T4 polynucleotide kinase was purified by electrophoresis on a 8% acrylamide gel, hybridised to oligo dT-cellulose purified hen RNA as above, and incubated with reverse transcriptase and the four deoxynucleoside triphosphates for 2 h at 37 °C. The elongated primer was run in lane 5 concomitantly with the sequencing reactions run in lanes 1 to 4, and after autoradiography yielded a single band (arrow head) indicating that the first nucleotide of the mRNA is the one just above the first U. The bands, which in lanes 1-4 are situated above the arrow head, correspond to elongation products of *Alu*1-*Alu*2 and *Pst*5-*Alu*1 fragments (see Fig. 1B(c)) which were present in the almost (but not fully) complete *Alu*1 digest of the *Pst*5-*Pst*6 fragment used to prime in the reactions run in lanes 1 to 4 (see above). (These bands were not seen when purified *Alu*3-*Alu*4 fragment was used as a primer.)

interesting to recall the absence of repetitive sequences for more than 17 kilobase pairs within and around the ovalbumin gene^{9,16,24}. The repetitiveness of the sequence which is common to *EcoRI* fragments 'b' and 'c' is unknown and the sequence might belong to the class of 15-fold repetitive sequences. It is interesting that in both cases they are situated close to highly repeated sequences. Current DNA sequence studies are further analysing these two classes of repeated sequences in an attempt to elucidate their relationship, if any.

Possible control regions at the 5' end of conalbumin mRNA and around the 5' end of the conalbumin gene

As for most eukaryotic mRNAs which have been sequenced up to now²⁵, the AUG codon located closest to the 5' end of con-mRNA is the one used to initiate translation. Although the mechanism for translation-initiation site selection in eukaryotes is still unknown²⁵, the striking conservation of the 3'-terminal sequence of 18S rRNA among eukaryotes led to the suggestion that this region of the 18S rRNA might base-pair with the complementary sequence of the initiation region in mRNA²⁶. Complementarity between the 18S rRNA sequence, 3'-UAGGAAGGCGU-5' and sequences of the 5'-non-coding regions of many eukaryotic mRNAs (see refs 25, 26 and refs therein), including ovalbumin mRNA^{24,27} (Fig. 7B), has been recognised. In the case of conalbumin, there are two such possible regions of complementarity (Fig. 7A), although both are shorter than for ovalbumin mRNA. It is striking that, like ovalbumin, one of these regions is immediately preceded by a sequence which can form a base-paired loop (nucleotides 2–29). Although the existence of a 5'-terminal loop is not a general feature of eukaryotic mRNA, there is a similar hairpin structure at the 5' end of SV40 VP1 mRNA²⁸. The possible role of such hairpin structures in the capping and/or in the translation-initiation processes remains to be established by site-directed mutagenesis.

A putative AT-rich promoter region (Hogness box) for RNA polymerase B (or II) has recently been proposed on the basis of a comparison of DNA sequences from several genes and is located 23–25 base pairs upstream from the base coding for the first nucleotide of the mRNA (see refs 24, 29 and refs therein). Similarly, a possible promoter site is also present 25 base pairs upstream from the conalbumin cap site (Fig. 7B). When compared to other putative promoter sites²⁴, it is apparent that in most cases the AT-rich region is flanked on both sides by GC-rich segments, indicating that the final length of this possible eukaryotic promoter sequence will probably be very similar to that of the extended Pribnow box³⁰. There is an amazing identity extending over 12 base pairs between the promoter sequence of the conalbumin gene and that of the major late adenovirus genes. In fact, this striking similarity is not limited to the Hogness box, but two additional regions of identity, 5 and 9 base pairs long, are found at positions –67 to –71 and –83 to –91, respectively. All of these identities, both in sequence and in position with respect to the cap site, cannot have occurred by chance and must correspond to common functional sites shared by these two evolutionarily distant genes. Again, site-directed mutagenesis studies will be required to investigate whether the regions of identity located upstream from the Hogness box could correspond to a site similar to the prokaryotic promoter recognition site³¹. In this respect, it is interesting that there are some similarities between the sequences present in these regions of the conalbumin and adenovirus late genes and sequences present in the corresponding regions of ovalbumin and other genes³².

On the other hand, although there are some additional similarities in the region corresponding to the first 250 base pairs upstream from the cap site when comparing the ovalbumin and conalbumin gene sequences^{24,32}, they are, surprisingly, much less impressive than those noted above for the conalbumin and adenovirus late major genes. This result could indicate that

there is no steroid hormone-mediated recognition of the DNA sequences present in this region. Alternatively, it may reflect the known difference of the ovalbumin and conalbumin genes in their hormonal response. Forthcoming sequence data for genes such as those for ovomucoid and lysozyme, and genes *X* and *Y* (refs 33, 34) whose hormone response is more like ovalbumin should be helpful in deciding between these two possibilities. Finally, although there is a striking similarity between the 10 residues which precede the cap site of adenovirus-2 late major and mouse β globin major genes^{35,36}, this homology sequence does not seem to be a general feature for eukaryotic messenger-coding genes, since it is absent from the conalbumin gene just as it is from other genes²⁴.

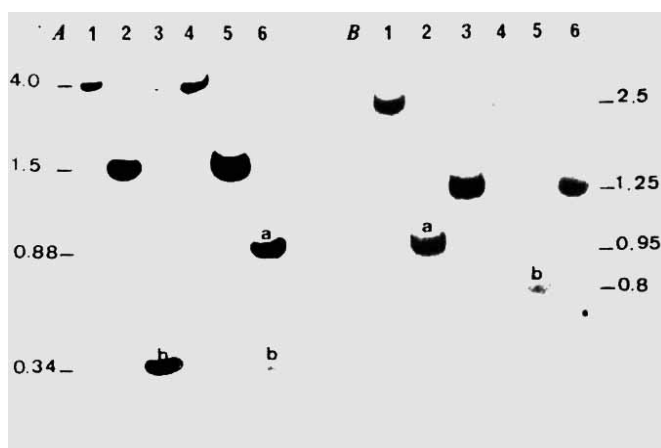


Fig. 6 Presence of repetitive sequences in the conalbumin *EcoRI* fragments 'b' and 'c'. A, Hybridisation of *EcoRI* fragment 'b' (lanes 1 and 4), and its *PstI* (lanes 2 and 5) or *MboII* (lanes 3 and 6) digests with *EcoRI* 'c' fragment (lanes 1–3) or total cellular chicken DNA (lanes 4–6) labelled with ³²P by nick-translation. B, Hybridisation of *EcoRI* fragment 'c' (lanes 1 and 4) and its *SacI* (lanes 2 and 5) or *PstI* (lanes 3 and 6) digests with *EcoRI* 'b' fragment (lanes 1–3) or total cellular chicken DNA (lanes 4–6) labelled with ³²P by nick-translation. The intact *EcoRI* 'b' (0.2 µg) or *EcoRI* 'c' (0.2 µg) fragments and their restriction enzyme digests were electrophoresed on a 2% agarose slab gel, transferred to nitrocellulose filters, hybridised with the labelled probes and revealed by autoradiography as described in legend to Fig. 2. Exposure time for autoradiography was 3 days for lanes 1–6 of panel A, 6 days for lanes 1–3 of panel B and 1 day for lanes 4–6 of panel B. a and b refer to hybridising fragments [see text and Fig. 1A(c), (d)].

Evolutionary and functional aspects of the organisation of the conalbumin gene

The conalbumin gene represents the most striking example of a split gene described to date. It is remarkable that, like the ovalbumin^{19,24}, ovomucoid^{37,38}, lysozyme^{39,40} and *X* and *Y*^{33,34} genes, all of the conalbumin exons are short with sizes ranging from 50 to 200 base pairs. Any attempt to explain the emergence and evolution of split genes should take into consideration the small size and the high number of exons in these genes. In particular, it seems difficult to accept the idea (see ref. 41 and refs therein) that these genes have evolved from already distinct exons, each coding for a different structural domain, and that they were brought together in the genome by random shuffling. It is noteworthy that, although con-mRNA is only 25% longer than ovalbumin mRNA, the mRNA-coding portion of the conalbumin gene is in 17 pieces, whereas the ovalbumin gene is in eight pieces. In this respect the conalbumin gene is more like the ovomucoid gene^{37,38} which possesses at least seven exons for an mRNA of about 800 nucleotides. We have learnt from the study of the ovalbumin and the ovalbumin-related *X* and *Y* genes^{33,34} that new split genes could have evolved by duplication of an ancestral gene which was already split in the same way. A similar conclusion was reached by analysing the structure of the α and β globin genes⁴². Such an origin of the conalbumin gene is

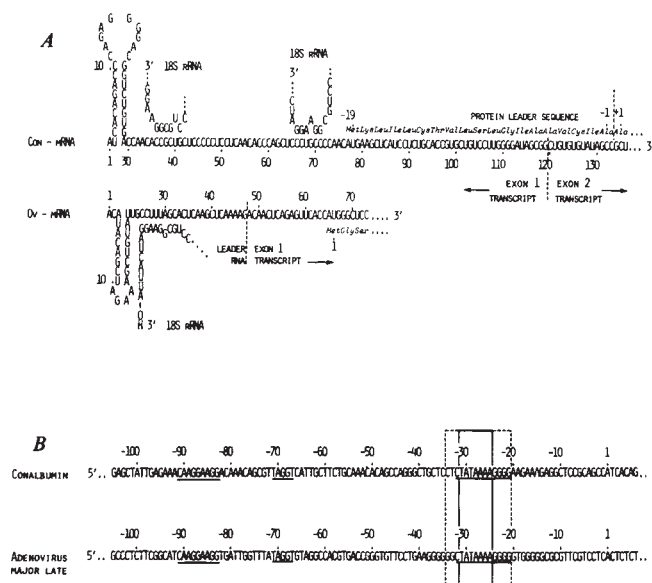


Fig. 7 A, Comparison between the 5' end region of conalbumin (con-mRNA) and ovalbumin (ov-mRNA) mRNA. The con-mRNA sequence was taken from Fig. 5A and the ovalbumin sequence from Fig. 5 of ref. 24. The complementarity of con-mRNA with two sequences present at the 3' end region of 18S rRNA is shown (see 'xt'). B, Comparison between DNA sequences (non-coding strands) of conalbumin and adenovirus-2 major late genes upstream from the regions coding for the 5' end of the mRNAs (position 1). The AT-rich and GC regions of homology are boxed with a continuous and a dashed line, respectively. The lines below the sequences indicate the regions of identity between the two DNA sequences.

unlikely, since we found no evidence for sequences cross-hybridising with the conalbumin exonic DNA in the chicken genome. However, the determination of the amino acid sequence of human and of chicken transferrins has provided very strong evidence that doubling of an ancestral structural gene occurred during the evolution of transferrins (refs 43–45 and J. Williams, personal communication). It therefore appears more likely that the present day conalbumin gene has evolved by duplication of an already split ancestor gene (perhaps in seven or eight pieces, like the present day ovomucoid gene). In this respect, the mechanism responsible for the appearance of the present day conalbumin gene could be similar to the duplication-fusion mechanism recently proposed by Sakano *et al.*⁴⁶ for the origin of the constant region of the mouse immunoglobulin $\gamma 1$ heavy chain, except that in the conalbumin case the

ancestral gene would already be a split gene. Clearly, the current detailed studies of the conalbumin gene structure should be very informative upon the question of how a new split gene may evolve by duplication of an ancestral split gene—a fundamental question, since gene duplication has perhaps been the predominant force involved in evolution⁴⁷.

It has been suggested that each exon might correspond to a functional or a structural domain (see ref. 41 and refs therein). Conalbumin provides an opportunity to test this idea, since the first 19 amino acids of pro-conalbumin belong to a functional domain which is involved in the secretion of the protein^{6,48}. Although it is not known which amino acids are absolutely required for the secretion of the protein, 14 out of the 19 amino acids which constitute the protein leader sequence are encoded in exon 1 (Figs 5A, 7A). A similar situation is encountered in the case of the mouse immunoglobulin light chain genes where 15 out of the 19 amino acids which constitute the protein leader sequence are encoded in exon 1^{49,50}. It remains to be seen whether this correspondence between exon 1 and the possibly functional portion of the protein leader sequence is coincidental or reflects a general feature of the organisation of genes coding for secreted proteins possessing a protein leader sequence. The existence of two homologous iron-binding domains in transferrins^{43–45,51} will provide an additional possibility to test the idea that exons could correspond to structural or functional domains.

Finally, the presence of repetitive sequences in intron C of the conalbumin gene is interesting in at least two respects. Firstly because, if the present day conalbumin gene has evolved by duplication of an ancestral split gene which was in seven or eight pieces, it is surprising that no repetitive sequences are found in the conalbumin *EcoRI* fragment 'a'. This absence might reflect the insertion of the repetitive sequences at a later stage during evolution or the loss of the duplicated counterpart. Secondly, the presence of these repetitive sequences should allow us to answer some questions related to their possible structural and/or regulatory role⁵².

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Hepatitis B virus genes and their expression in *E. coli*

Mark Pasek*, Tadaatsu Goto*, Walter Gilbert*

* The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Barbara Zink†, Heinz Schaller†

† Department of Microbiology, University of Heidelberg, Im Neuenheimer Feld 230, 6900 Heidelberg, FRG

Patricia MacKay‡, Glynis Leadbetter‡ & Kenneth Murray§

‡ Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh, UK

§ Department of Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh, UK

A composite DNA sequence of regions of hepatitis B virus, determined from a series of recombinant plasmids, reveals the genes for the surface antigen and the core antigen of the virus. The sequence of the core antigen shows it to be a DNA binding protein. The core antigen gene is expressed in Escherichia coli and when injected into rabbits the bacterial product induces antibodies which react with core antigen isolated from human sources.

DNA FROM hepatitis B virus (HBV) of three different serotypes has been cloned in *Escherichia coli* via phage λ derivatives^{1,2} and plasmids^{3,4}. Some of the transformed cells produce an antigen that reacts specifically with hepatitis B core antibodies (HBcAb)⁵; these cells contain hybrid plasmids, constructed by insertion of HBV DNA fragments into pBR322 at the *R.Pst*I cleavage site via 3' oligo (dG) and oligo (dC) tails to facilitate the expression of viral antigen genes as polypeptides fused to β -lactamase⁵. Thus, the coding sequence of the HBV core antigen should be adjacent to or near the point of fusion to the β -lactamase gene. Nucleotide sequence analysis might identify HBV genes since the molecular weights of the core antigen (HBcAg) and surface antigen (HBsAg) are known^{6,7}, as are partial amino acid sequences from the N- and C-termini of HBsAg⁸.

DNA fragment mapping and nucleotide sequence determination

The cloning of HBV DNA fragments isolated from blood plasma with the complex serotype *adyw* was described previously³, together with some properties of some of the recombinant plasmids. The viral DNA has an unusual structure: there is a nick in one strand and a gap in the other⁶. Table 1 lists the plasmids chosen for sequence analysis and shows the sizes of the HBV fragments that they contain. All those plasmids imparting

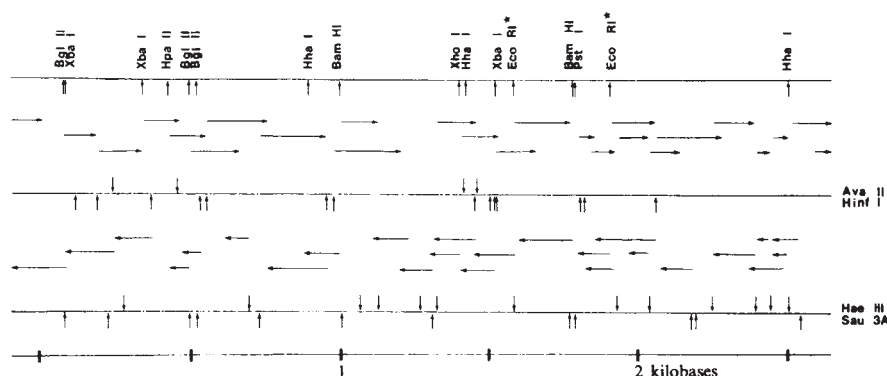
Table 1 HBV clones

HBcAg positive					
Clones	Ala ₁₈₂	Left endpoint	Phe	His	Right endpoint
pHBV64	GCA	(GGG) ₅ GTT	TTT	CAC	1,788
pHBV66	GCA	(GGG) ₆ GAA CTT	TTT	CAC	~1,710
pHBV139A	GCA	(GGG) ₈ GGA CTT	TTT	CAC	~1,260
pHBV110	GCA	(GGG) ₇ CTT	TTT	CAC	~1,240
	GCA	(GGG) ₆ GGC	TTT	TTC	
pHBV138	GCA	(GGG) ₅ CTT	TTT	CAC	1,842
pHBV139	GCA	(GGG) ₇ CTT	TTT	CAC	1,625
HBcAg negative					
Clones	Left endpoint		Right endpoint		
pHBV15	1,967		2,656		
pHBV20	1,447		2,199		
pHBV114	~-80		~2,270		

The structure of the oligo (dG) stretch that fuses to Ala₁₈₂ of β -lactamase is given for the HBcAg producing plasmids. Clone pHBV110 has two plasmids, one out of phase by one base.

to their host cell the ability to synthesise core antigen, as detected in the solid phase radioimmunoassay⁹, contained HBV sequences in the same orientation; in some cases this was confirmed by heteroduplex electron microscopy. Fragments were prepared from these plasmids by digestion with appropriate restriction enzymes and labelled at their 5' termini with [γ -³²P]ATP and T4 polynucleotide kinase, and their nucleotide sequences were determined by the chemical degradative methods of Maxam and Gilbert¹⁰. Figure 1 outlines the sequencing strategy and Fig. 2 shows the composite sequence. The sequence shown covers about 87% of the virus; our set of clones has proved deficient in representations of the region of the gap in the viral DNA.

Fig. 1 The positions of restriction targets in HBV DNA and the strategy for nucleotide sequence determination. For simplicity the restriction targets are distributed between three maps, but each covers the same DNA sequence which, like the sequence shown in Fig. 2, begins at the point of attachment to the *Pst*I site in pBR 322. Vertical arrows indicate the positions of restriction targets and the horizontal arrows show the direction and extent of the sequencing runs; the sequencing techniques were the chemical methods of Maxam and Gilbert¹⁰.



[illegible]

Fig. 2 The nucleotide sequence of HBV DNA shown as fused into pBR322 and expressing HBcAg (the first phase). The major possible translation products in all three reading frames are shown starting at the first ATG after a terminator^{***}. The sequence is numbered from the A of the ATG for initiation of the core antigen as reference. Core antigen sequence runs from residues 1 to 549; the surface antigen is encoded between residues 1,437 and 2,114. The sequence was derived principally from clone pHBV138 and was extended by results derived from pHBV20 and pHBV15. The arrow shows the beginning of the sequence coding for HBV surface antigen. Several shorter translation products are possible, but have been omitted from the figure for simplicity.

The core antigen gene

All of the recombinants examined that produced HBcAg proved to have the HBV DNA insertion in the same phase with respect to the R.PstI site in the β -lactamase gene beginning within one or two bases of the same sequence. Table 1 lists the structure of these junctures. Since the original HBV fragments came from digests with different restriction enzymes, R.BamHI and R.KpnI, this unique attachment was surprising. It may have resulted from inadvertent breakage, or tailing, of the DNA (from Dane particles) at the nick in the molecule⁹. We expected that the core antigen, in whole or in part, would be fused to β -lactamase via a small number of glycine residues, but that is not the case. In six strains that produce antigen, the β -lactamase is fused through 5 to 8 glycines to the same peptide sequence, but this sequence terminates 25 amino acids further on. In this phase the stop codon is followed three nucleotides later by an initiation sequence from which translation continues unhindered to give a polypeptide of 183 residues (Fig. 2, positions 1 to 549). Stop codons are abundant in the other two translation phases throughout this region.

Because there is a requirement for in-phase reading of this termination signal, the situation resembles the restart phenomenon observed in bacterial proteins. We conclude that the core antigenic activity resides in a polypeptide of molecular weight 21,000 translated *de novo* from the transcript of the viral DNA sequence. This product would probably remain within the cell, rather than be within the periplasmic space; this is consistent with our observations that HBcAg activity is only detected in bacterial extracts, not in osmotic 'shockates'.

The core antigen

Amino acid sequences for the *in vivo* core antigen have not been published, but the calculated size of the antigen resulting from translation of the nucleotide sequence carried in the bacterial plasmid is close to the molecular weight of 19,000 observed for the antigen isolated from Dane particles^{6,7}. We do not know if any amino acids have been trimmed from the amino terminus of the authentic protein. The deduced polypeptide sequence is interesting; while the protein is not particularly remarkable in its amino-terminal half, sequences homologous with protamines and especially with galline¹¹ (from rooster sperm) are found towards the carboxy terminus (residues 150–183, nucleotides 448–549). In this region 16 out of 34 amino acids are arginines, arranged in groups of 3 or 4 separated by 4 other residues (Fig. 2). The sequence Ser-Pro-(Arg)₃ occurs three times, while there is a double repeat of the sequence Ser-Pro-(Arg)₄-Ser-Gln. The obvious inference is that the C-terminal region binds the HBV DNA within the Dane particles, while the rest of the protein participates in other structural roles. The four cysteine residues offer the possibility of internal disulphide bridges as well as intermolecular interactions, the latter possibly explaining some of the higher molecular weight values observed for HBcAg⁶.

Although the structure of the messenger for HBcAg is unknown, we support the inference that the ATG at position one is the initiator by noting a secondary structure feature in the DNA sequence, and thus in a hypothetical mRNA, just before that ATG: a stem with a loop that could pair with the 18S ribosomal RNA analogous to structures that have been found in preproinsulin messages and some other higher cell mRNAs^{12–14}. Figure 3 shows this feature. Although short oligonucleotide sequences that would possess a similar capacity for such interactions can be found elsewhere in the HBV DNA sequence, none can be related to an initiation codon in a simple way.

In vivo activity of the core antigen produced from *E. coli*

To test the biological activity of the antigenic polypeptide translated from the plasmid transcript, sterile extracts of the bacterial cells mixed with an equal volume of Freund's adjuvant were injected into rabbits. Two animals received the crude bacterial extract, and two were given samples of the same extract

Table 2 Amino acid composition for HBsAg

Serotype:	adw*	ayw3*	ayw†	adyw‡
Total residues:	200	200	220	226
Ala	7	10.2	7	6
Asp	11.2	13.4	11	3
Asn	—	—	—	5
Arg	4.4	6.0	7	6
Cys	14.4	10.2	—	14
Glu	11.6	15.2	10	2
Gln	—	—	—	7
Gly	12.1	18.4	19	15
His	1.4	1.0	1	1
Ile	11.4	10.8	11	17
Leu	22.4	30.8	36	35
Lys	4.4	3.8	3	2
Met	6	6.2	6	6
Phe	10	12.6	15	16
Pro	25	24.8	26	21
Ser	23.8	28.0	25	26
Tyr	4.8	5.2	5	6
Thr	16.8	21.0	21	16
Trp	—	—	—	13
Val	8.2	10.6	11	9

* From Peterson *et al.*²¹.

† D. L. Peterson, personal communication.

‡ This work.

after fractionation on a Sephadex G-50 column. Additional injections of the same sample, which had been stored frozen and shown to retain full antigenic activity, were given 2 weeks and 5 weeks after the first injection, and the animals were bled at intervals several weeks after the initial injection. Figure 4 shows the results of immunodiffusion experiments¹⁵ comparing these sera to human HBcAb (HBcAg positive), using HBcAg derived from human liver¹⁶. All four rabbit sera gave precipitin lines with HBcAg identical to those formed between this antigen and HBcAb derived from human sources. Thus, the core antigen synthesised in *E. coli* is serologically active *in vivo*. Regardless of whether antibodies to this particular antigen confer protection against HBV *in vivo*, the experiment does establish the feasibility of vaccine production from viral antigens synthesised in microbial cells.

The surface antigen gene

None of the hybrid plasmids used in the sequence analysis synthesised any surface antigen that could be detected by the radioimmunoassay. However, amino acid sequences of parts of HBsAg are known⁸ so that a corresponding gene sequence could be sought along the DNA. Figure 2 shows such a sequence, starting at residue 1,437, which runs from the observed N

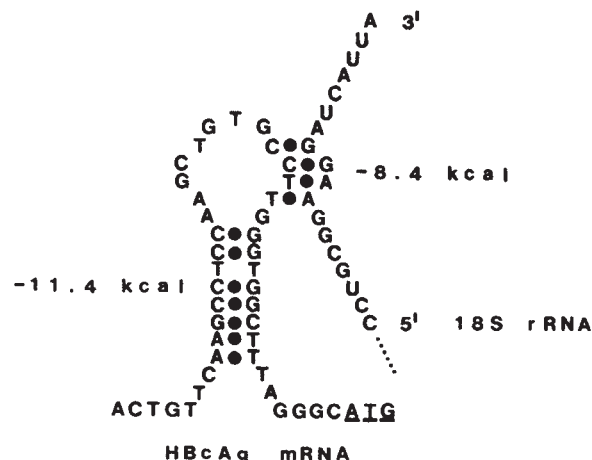


Fig. 3 A secondary structure feature lying immediately before the initiator of the core antigen which could pair with the 18S rRNA.

Table 3 Hepatitis B sequence heterogeneity

	pHBV138	pHBV64	pHBV139	pHBV110	pHBV20	pHBV170
Gene/position of initial nucleotide						
HBcAg/376	ATT Ile					GTT Val
HBcAg/424	ACG Thr	ACA Thr				
(pre?)HBsAg/1,236	CTG Leu			GTG Val		
(pre?)HBsAg/1,431	CTG Leu	CGG Arg				
HBsAg/1,575	GTG Val	GTG Val	GCG Ala		GTG Val	
HBsAg/1,623	ACC Thr		AGG Arg		ACC Thr	
HBsAg/1,764	ATT Ile				CTT Leu	

terminus, Met-Glu-Asn-Ile-Thr-Ser- (ref. 8) to a terminator 226 amino acid residues away. We presume that this protein is initiated at the ATG at residue 1,437, but we cannot exclude the possibility that the mature protein is cleaved from a larger polypeptide initiated at the ATG at residue 948, since there is no termination codon between these two triplets. This polypeptide sequence, corresponding to a protein of molecular weight 25,400, also contains two peptide sequences of nearly identical composition to those for two amino-ethyl-cysteinyl peptides of 13 and 28 amino acids in length isolated after digestion of HBsAg with trypsin (D. L. Peterson, personal communication), as well as the correct C-terminal dipeptide⁸, all in the same reading frame. The amino acid composition of HBsAg deduced from the nucleotide sequence is in accord with the results of analysis of the antigen itself (Table 2). The molecular weight of HBsAg in the glycosylated form has been estimated to be 25,000, while that of the nonglycosylated form of this polypeptide, which is also immunogenic, has been estimated to be 22,000^{7,8}. There is one characteristic glycosylation sequence in this protein, the Asn in the third position, which lies in a ... Asn-Ile-Thr-Ser ... sequence¹⁷. The location in the cloned HBV DNA of a nucleotide sequence corresponding with amino

acid sequences of HBsAg is strong evidence that the surface antigen is a viral gene product and not a host gene product induced by infection with the virus. The donor of the plasma used as a source of Dane particles for these clonings showed a complex serotype, a mixture of *adw* and *ayw*, and presumably was infected by two viruses. We have observed micro-heterogeneity between different clones. Table 3 lists these sequence variations (not exhaustively). Several of the variations are silent, or not in known products, but three changes occur in HBsAg, substitution of Ala for Val and Arg for Thr between clones pHBV138 and pHBV139 as well as a change of a Leu for Ile in a third clone, and these could reflect this complex serotype. The sequence heterogeneity of the different clones demonstrates that the infection involves a heterogeneous virus population. Furthermore, the gene for surface antigen extends into the region of the gap in the HBV DNA molecule and may be drifting rapidly.

The nucleotide sequence of the recombinant plasmids shows that none of those examined has the HBsAg gene in a position from which we would expect any biologically active polypeptide to be produced, and no HBsAg was detected. The sequence results immediately indicate subcloning experiments for the expression of the surface antigen as a polypeptide fused to β -lactamase. Some of the clones obtained from these experiments have given positive reactions in the radioimmunoassay for HBsAg.

Other HBV genes

In addition to the core and surface antigens, the *e* antigen (HBcAg) and the endogenous DNA polymerase of the Dane particle should be encoded in the viral DNA; however, little is known of their properties. The DNA sequence suggests that the viral information is encoded in overlapping readings in one direction. The largest translation product encoded anywhere by the opposite DNA strand is 62 amino acids, while if the HBV nucleotide sequence is read in the third reading frame (with respect to core and surface antigen sequences), a translation product overlapping the C-terminal region of the core antigen as well as the entire gene for surface antigen continues for at least 750 amino acid residues (to nucleotide 2656 which is the extent of the sequence so far determined) and so could perhaps be a candidate for the DNA polymerase. Bacterial and viral DNA polymerases have molecular weight in the range 109,000–120,000^{18,19}, whereas that of the *e* antigen is probably around 19,000²⁰.

In this discussion we have assumed for simplicity that there is no important splicing of the viral RNA within the translated regions of the genome. However, a full understanding of the mechanism of expression of the viral genes must await an identification of the structure of the viral messages. Now that cloned fragments and DNA sequences of HBV are available, a complete unravelling of the molecular biology of this human disease is possible.

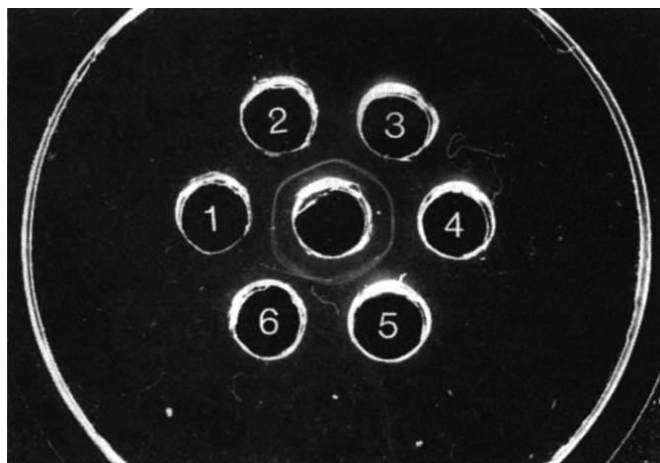


Fig. 4 An Ouchterlony immunodiffusion experiment comparing rabbit anti-(*E. coli*-HBcAg) to human anti-HBcAg in a 0.9% agarose gel in 0.01 M Tris-HCl pH 7.2, 0.001 M EDTA, 0.1M NaCl. The centre well contained partially purified HBcAg derived from human liver¹⁶. Wells 1 and 4 contained human HBcAb at a dilution of 1 in 30, wells 2 and 3 contained undiluted serum from rabbits injected with the unfractionated extract from bacteria carrying clone pHBV139, and wells 5 and 6 contained undiluted rabbit antiserum raised against HBcAg from the same bacterial extract, but fractionated on a column of Sephadex G-50. Equivalent results were also obtained in a solid phase radio-immunoassay.

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sequence given here differs in 15 positions from that in ref. 23 and shows 30 base changes from that in ref. 22. However, the three sequences are highly conserved in the regions corresponding with the N- and C-terminal domains of the surface antigen, while appreciably more than half of the base changes occur within the central region of the protein, many of them producing a change in amino acid. Sequences flanking the coding region are included in ref. 22 and much more difference from the sequence published here occurs in these flanking regions; some 15% of the bases differ in the region ahead of the coding sequence and about 11% in the region following it, compared with an average of 4% difference between the two sequences in the coding region.

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Equilibrium: the intracellular distribution of steroid receptors

Peter J. Sheridan, James M. Buchanan & Vincent C. Anslemo

Department of Anatomy, University of Texas, Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284

Pierre M. Martin

Laboratoire Récepteurs Hormonaux, Institut Paoli Calmette, Marseilles, France

Data are presented which suggest that there are unbound receptors for oestrogen in nuclei of the smooth muscle cells of the myometrium. A new model for the distribution of unbound receptors is proposed in which unbound receptor is in equilibrium, partitioned between nucleus and cytoplasm according to the free water content of these intracellular compartments.

THE most widely accepted model for the intracellular translocation of steroids was developed from many studies concerning the uptake and retention of oestradiol in cells of the female reproductive tract. According to this model, oestradiol enters the uterine cells, binds to a specific high-affinity cytoplasmic receptor and is then translocated to the nucleus by a temperature sensitive process. During this process, the oestrogen receptor undergoes a conformational change which is discernible by an increase in its sedimentation coefficient from 3.8–4S to 5–5.2S and by its ability to bind to isolated uterine nuclei and stimulate RNA synthesis^{1–3}. However, there are data in the literature which question the validity of this model (for review see ref. 4). Briefly, free receptor and 4S receptor have been found in carefully washed nuclei^{5–9}, and the conversion of 4S to 5S has been shown to take place more rapidly in the presence of DNA¹⁰. Much of the support for the classical model for the intracellular translocation of steroids comes from autoradiographic studies conducted together with the early biochemical studies¹¹. After a 1-h incubation at 37 °C the steroid was localised autoradiographically in the nucleus, agreeing with the biochemical data, whereas after a 5-min incubation at 2 °C the steroid was localised autoradiographically outside the nucleus.

Close examination of the autoradiogram shown¹¹ does not reveal a cytosol concentration of steroid but rather a diffuse pattern resembling background. This interpretation does not agree with the corresponding biochemical data¹¹. Because we were unconvinced by the autoradiogram and because in our hands whole uteri are subject to problems associated with diffusion and washing, we initiated experiments which we feel demonstrate unbound nuclear receptors in rat uterus and a breast tumour line. In addition, we propose a new model for the intracellular distribution of steroid receptors in which unbound receptor is in equilibrium, partitioned between cytoplasm and nucleus according to their respective free water content.

Autoradiographic studies: nuclear receptor at 4 °C

The immature rat uterus was studied in detail because it was used by the original investigators to establish the current model for the mechanism of action of steroids. A second system was used to confirm these results—cells from a breast tumour line designated MCF-7.

Whole uteri from 21–23-d-old rats (Sprague–Dawley) were incubated in minimal essential medium (MEM, GIBCO) in the presence of 5×10^{-9} M [$1, 2, 6, 7$ -³H]oestradiol-17 β (89 Ci mmol⁻¹, Amersham/Searle) with or without 5×10^{-7} M unlabelled oestradiol (E₂, Sigma) for either 5 min or 2 h at either 0 or 37 °C. Identical incubations were carried out with ³H-R2858([$6, 7$ -³H]methoxy-17 ethyloestradiol, 52 Ci mmol⁻¹) or with ³H-R2858 + unlabelled R2858. The tissue was then processed for autoradiography¹² to determine the location of the ³H-steroid. Additionally, some tissues were incubated for 5 min at 0 °C and processed biochemically (see below). Nuclear local-

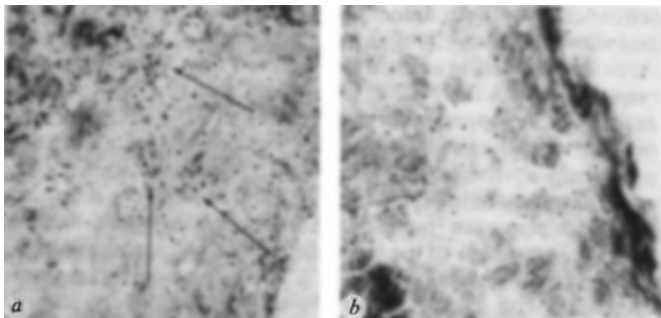


Fig. 1 Autoradiograms of rat uteri from the extreme periphery of the sections. The serosa appears to the right in both autoradiograms. *a*, Incubated at 0°C for 5 min with ^3H -oestradiol-17 β ($5 \times 10^{-9}\text{M}$), *b*, incubated at 0°C with ^3H -oestradiol-17 β ($5 \times 10^{-9}\text{M}$) + unlabelled oestradiol-17 β ($5 \times 10^{-7}\text{M}$). Note the nuclear localisation (arrows) of silver grains over myometrial cell in *a* and the lack of grains over nuclei in *b*. Stained with methylgreen-pyronin, 40-d exposure, 4- μm section, 500 $\times$.

isation was found at 0°C at both 5 min (Fig. 1) and 2 h (not shown) in the myometrium at the extreme periphery of the section. Autoradiograms from uteri incubated at 0°C indicated that there was little diffusion of the ^3H -steroid even after the 2-h incubation (Fig. 2), and thus the inner portion of the myometrium and the endometrium were unlabelled. Also at neither time was there any indication of an exclusive cytoplasmic localisation of ^3H -steroid. Nuclear localisation was also found at 37°C at 5 min and 2 h (not shown). We repeated these experiments three times with ^3H -oestradiol and three times with ^3H -R2858 (Fig. 3) and obtained identical results.

Our autoradiographic localisation of a nuclear concentration of ^3H -E₂ and ^3H -R2858 after 5 min of incubation at 0°C is in direct conflict with the earlier autoradiographic results¹¹. The reason(s) the earlier workers did not see nuclear localisation is (are) unclear. Possible explanations are the differences in specific activities (earlier work 57.4 cm mmol⁻¹ compared with our 89 Ci mmol⁻¹) and exposure time (in the earlier work localisation was found in 45 d with dry-mount technique while in our work the earliest localisation was found at 30 d with the thaw-mount technique, which is usually two to three times faster than the dry-mount technique).

MCF-7 cells were also incubated in MEM in the presence of different concentrations of ^3H -E₂ or ^3H -R2858 (5×10^{-11} – 10^{-7}M) alone or in the presence of 100-fold unlabelled E₂ for 2 min–2 h at 0°C and 37°C. After incubation the MCF-7 cells were washed three times in MEM, then washed with an antibody generated against E₂ (Roussel ACE₂-3341 diluted 1/500 in MEM) and then spun down in 0.3 M sucrose (MEM). The use of antibody against E₂ greatly reduced background. The pellet of cells was then mounted on a stud with OCT (Tissue-Tek II), frozen in liquefied propane and processed for autoradiography.

After 2 h of incubation at 0°C, approximately 76% of the grains were located over the nucleus (Fig. 4), whereas at 37°C more than 90% were over nuclei. Nuclear localisation was found at 0°C with as little as 2-min of incubation. The ratio of grains over the nucleus and cytoplasm over the concentration of ^3H -ligand used remained constant. However, at the lower concentrations of ligand and the amount of exposure necessary to observe localisation increased that is, the quantity of observed silver grains was dose dependent. This confirms the biochemical studies of nuclear localisation of receptor at 0°C in MCF-7 cells⁷.

Biochemical studies: problems with loosely trapped steroid

Autoradiograms from rat uteri exposed to ^3H -steroid at 0°C for 5 min and 2 h, showed that a 5-min incubation at 2°C would not result in the filling of 60–75% of the cytoplasmic receptors, as

originally reported¹¹. To resolve the discrepancy between the autoradiography in our study and the previous biochemical data, we investigated whether the binding observed previously¹¹ could have been the result of steroid–receptor interactions after the initial incubation period, that is during centrifugation at 225,000g for 1 h. In our first experiment uteri were incubated for 5 min at 0°C with ^3H -E₂ ($5 \times 10^{-9}\text{M}$) or ^3H -E₂ ($5 \times 10^{-9}\text{M}$) + unlabelled E₂ ($5 \times 10^{-7}\text{M}$). After 5 min the uteri were washed three times with buffer and then homogenised. In a second group, ^3H -E₂ (5 nM) was added to the uteri, and they were homogenised immediately (0.1 M PO₄, 0.01% monothioglycerol, 5 mM Mg and 10% glycerol, pH 7.4 at 0°C). The homogenates were then spun at 105,000g for 1 h, and the resultant supernatants (cytosol) were withdrawn for either a hydroxyapatite assay or sucrose gradients (see below). Protein concentrations were determined by the method of Lowry¹³.

For the hydroxyapatite assay¹⁴, 200 μl of cytosol was mixed with 200 μl of hydroxyapatite suspension (1 volume dry powered DNA-grade hydroxyapatite (Bio-Rad) made up to 2 volumes with buffer). Tubes were mixed frequently for 30 min and then centrifuged for 2 min at 800g. The supernatant was discarded and the hydroxyapatite was washed three times with 2 ml of buffer with the same centrifugation each time. Then 1 ml of 100% ethanol was added to the hydroxyapatite and the mixture was incubated at room temperature for 1 h with frequent remixing to extract ^3H -steroid. Tubes were then centrifuged for 2 min at 200g and the supernatant was counted with 5 ml of ACS scintillation fluid (Amersham/Searle).

For the sucrose gradients, 200 μl of cytosol were layered on a density gradient of 5–30% sucrose in buffer. The gradients were centrifuged at 54,000 r.p.m. on a Beckman SW60 rotor for 16 h. Fractions of 200 μl were collected from the bottom and counted with 5 ml of ACS scintillation fluid.

In both the hydroxyapatite and the sucrose density gradient assay we obtained specific binding (8S) in cytosols from tissue which had been incubated with 5 mM ^3H -E₂ and in those not labelled until just before homogenisation (data not shown). Labelling with 5 nM just before homogenisation resulted in a fourfold increase over that seen in the tissue that was incubated and then washed.

In a second experiment we varied the concentration of the ^3H -E₂ added just before homogenisation and found that 1.25 nM added just before homogenisation was equivalent to a 5-min incubation with 5 nM. Similar results were obtained with the hydroxyapatite.

In a third experiment we prepared cytosol by centrifugation at 250,000g and did not add labelled steroid until just before centrifugation (that is, after homogenisation). Again we obtained as 8S peak indicating that the steroid–receptor interaction took place in the centrifuge. Therefore, ^3H -E₂ can bind to free receptor in the centrifuge tube. Thus, any loosely trapped steroid outside the target cells of standardly washed uteri could bind to free receptor during the tissue homogenisation and the

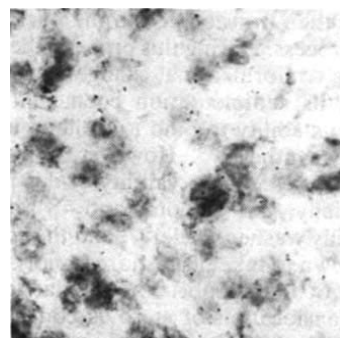


Fig. 2 Autoradiogram of rat uteri from the centre of a section incubated for 2 h at 0°C with ^3H -oestradiol-17 β . Note the paucity of silver grains. Stained with methylgreen-pyronin, 40-d exposure, 4- μm section, $\times 500$.

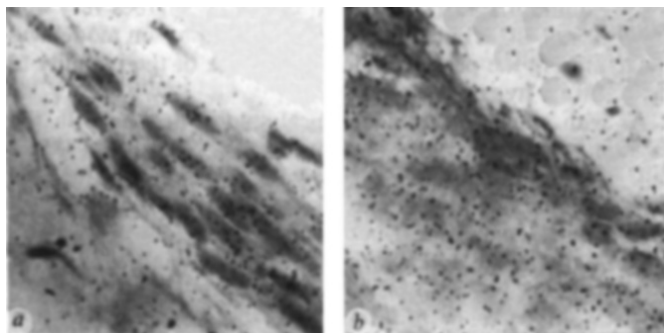


Fig. 3 Autoradiograms of rat uteri from the extreme periphery of the sections. The serosal surface is to the right in both autoradiograms. *a*, Incubated at 0 °C for 5 min with ^3H -R2858 ($5 \times 10^{-9}\text{M}$); *b*, incubated at 0 °C with ^3H -R2858 ($5 \times 10^{-9}\text{M}$) + unlabelled R2858 ($5 \times 10^{-7}\text{M}$). Stained with methylgreen-pyronin, 3.5-month exposure, 4- μm section, $\times 500$.

preparation of cytosol in accord with the demonstration¹⁵, with the use of cold chase experiments, that most of the binding reported earlier¹¹ occurred after the initial incubation period. The autoradiographic data presented here and the early biochemical data¹⁵, together with our biochemical data therefore indicate that most of the binding of ^3H -E₂ to the receptor reported earlier¹¹ can be accounted for not in the incubation period, but during preparation of the cytosol. In addition, our autoradiographic data point to a nuclear localisation of ^3H -E₂ or ^3H -R2858 in the smooth muscle cells at the periphery of uteri incubated for 5 min at 0 °C, thus suggesting the presence of unbound nuclear receptor. The only evidence for the two-step cytoplasmic model for the intracellular translocation of steroids in the rat uterus is the observation that on homogenisation in a buffer most of the binding activity appears in the cytosol.

Equilibrium: mechanism of action of steroids

One mechanism which would explain the appearance of all binding activity in the cytosol after homogenisation of untreated uteri (when much of it was of nuclear origin *in vivo*) is a change in the free water content of the cytoplasmic compartment. That is, if the receptor were in equilibrium between the nucleus and cytoplasm, partitioned in some way according to the free water present in each compartment, homogenisation of uteri in buffer would artificially increase the water content of the non-nuclear compartment, that is, the cytosol, in agreement with the water equilibrium model¹⁶. To reestablish the original equilibrium much of the nuclear receptor would have to enter the cytosol. We tested this hypothesis with the following experiments.

Twenty-four uteri from 21–23-d-old Sprague–Dawley rats were homogenised at 0 °C in 3.5 ml of phosphate buffer (5 mM sodium phosphate, 0.01% mM monothioglycerol, 10% glycerol, pH 7.4) or Tris buffer (0.01 M Tris, 0.0015 M EDTA, 0.01% monothioglycerol, 10% glycerol, pH 7.4). Aliquots of the homogenate were distributed to six tubes and the following volumes of buffer were added to the tubes: 0, 0.5, 1.5, 4.5, 13.5 and 40.5 ml. The tubes were allowed to stand at 0 °C for 5 min and then centrifuged at 800g for 10 min. The resultant supernatants were decanted off. The first three tubes containing the supernatant were brought to a volume of 3 ml with buffer to insure an adequate volume of cytosol for the assay and all supernatants were then spun at 105,000g for 1 h. The resultant supernatants (cytosols) were then assayed for oestrogen receptors (see below). The nuclei from the 800g spin were extracted with either 3 ml of buffer or 3 ml of 0.6 M KCl in buffer for 60 min at 0 °C and then centrifuged at 800g. The supernatants were decanted and assayed for oestrogen receptor. For reasons that will become obvious it was considered imperative that the nuclear preparation remain as crude homogenates.

Samples of 200 μl of cytosol or KCl extract or buffer extract of nuclear pellet were incubated with 50 μl of $[1,2,6,7\text{-}^3\text{H}]\text{E}_2$ (109

Ci mol⁻¹, final concentration 5×10^{-9}) alone or with ^3H -E₂ + 5×10^{-7} unlabelled E₂ for 3 h at 0 °C. After incubation, the tubes were assayed by the hydroxyapatite method¹⁴.

Figure 5 shows the effect of adding increasing volumes of buffer before separation of cytosol from the nuclear fraction (note that all values were converted to total amount of receptor in either nucleus or cytoplasm). Increasing the amount of buffer decreases the amount of receptor in the nucleus. We have repeated this experiment seven times with similar results. The shape of the curve is highly suggestive of a simple equilibrium or of a desorption of receptor from the nuclear pellet (the autoradiographic data suggest that some receptor is of nuclear origin).

We assume the following model. Nuclear receptors (R_n) are in equilibrium with cytoplasmic receptors (R_c) *in vivo*. The equilibrium may be represented by a distribution coefficient identifying the components of the partitioning of the receptors between the nucleus and the cytoplasm.

$$R_c \leftrightarrow R_n \quad (1)$$

and

$$D = \frac{R_n/V_n}{R_c/V_c} \quad (2)$$

where V_n and V_c are the nuclear and cytosol fluid volumes, respectively. Introducing $R_T = R_n + R_c$ and solving this equation for R_n yields:

$$R_n = \frac{R_T}{V_c K + 1} \quad (3)$$

where

$$K = \frac{1}{DV_n} \quad (4)$$

The limits of equation (3) indicate the direction of flow of the receptors between nucleus and cytoplasm. As $V_c \rightarrow 0$, $R_n \rightarrow R_T$

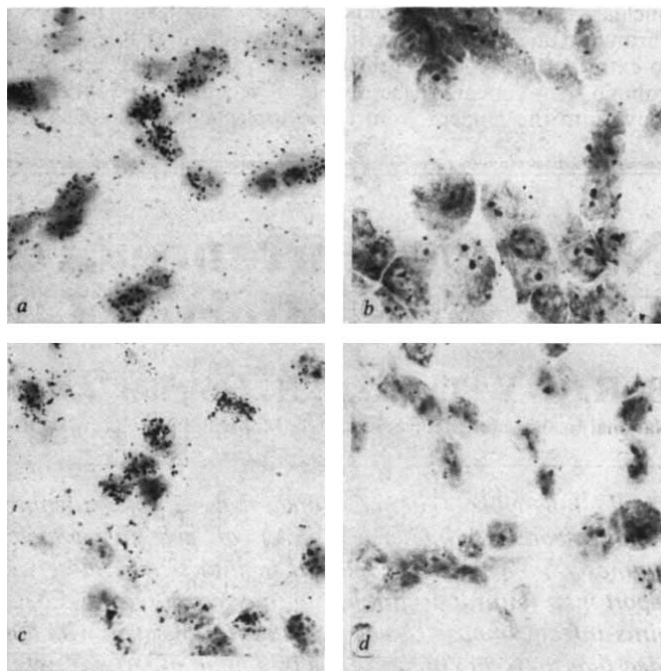


Fig. 4 Autoradiograms of MCF-7 cells. *a*, Cells incubated for 2 h with ^3H -oestradiol ($1 \times 10^{-9}\text{M}$) at 0 °C, and *c*, at 37 °C. *b*, Cells incubated for 2 h with ^3H -oestradiol ($1 \times 10^{-9}\text{M}$) + unlabelled oestradiol ($1 \times 10^{-7}\text{M}$) at 0 °C, and *d* at 37 °C. The predominant localisation is nuclear at both 0 °C and 37 °C. The large black dots seen in the nuclei of unlabelled cells (*b* and *d*) are nucleoli. Stained with methylgreen-pyronin, 2-week exposure, 4- μm sections, $\times 900$.

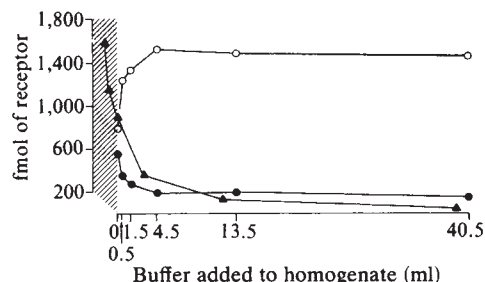


Fig. 5 Amount of apparent nuclear receptor (●), and amount of apparent cytoplasmic receptor (○) plotted as a function of volume of buffer added to homogenate. At 0 added volume the slope of the curve is quite steep. The theoretical values for nuclear receptor that would be obtained if $R_T = 1,588$ fmol and $K = 0.75$ in equation (3) are plotted as a function of the cytosolic volume (V_c) (▲). Because we can never approach $V_c = 0$ or the *in situ* volume of the cytoplasm experimentally, we have moved the x-axis to the right an arbitrary amount and put this part of the theoretical curve under cross-hatching.

(*in vivo*) and as $V_c \rightarrow \infty$, $R_n \rightarrow 0$ (in typical *in vitro* experiments in which large volumes of buffer are used for homogenisation). R_n is measured as c.p.m. in the nuclear pellet and V_c is the total added volume of the homogenate. In Fig. 5 we plot R_n and R_c as a function of V_c . The third plot in Fig. 5 contains points calculated from equation (3) with $K = 0.75$.

For the immature rat uterus, the distribution constant is high. We expect that this constant will vary with different steroids and different tissues. This model provides for the first time a mechanism for the translocation of cytoplasmic (*in vivo*) or cytosolic (*in vitro*) receptor to the nucleus (no mechanism has been found which is compatible with the two-step cytoplasmic model¹⁷).

In this scheme, free steroids enter both the cytoplasm and the nucleus of a target cell and bind to unoccupied receptors. *In vivo* or *in vitro* at 37 °C, the receptors are activated *in situ* and the nuclear fraction of steroid-receptor complexes binds to chromatin and thus precipitates out of solution (that is, one has to extract with KCl to get them back in solution). The nuclear volume then appears to be devoid of receptors and receptors move into the nucleus from the cytoplasm to reestablish the

initial equilibrium (translocation). This model agrees very well with the published data, and in fact the equilibrium model was considered in the past but rejected because of the inability to demonstrate free nuclear receptor¹⁸.

As with many of the biochemical studies dealing with the location of unbound steroid receptors¹⁵, our data are subject to different interpretations. Alternative explanations for the results of our dilution experiments are (1) that the initial homogenisation of the tissue in hypotonic buffer caused swelling of the nuclei and an initial shift of cytoplasmic receptor into the nuclear compartment and that subsequent dilutions just redistributed the receptor back into the cytoplasmic compartment, or (2) that the increased nuclear receptor with a concentrated homogenate is due to nonspecific adsorption of cytoplasmic receptor to the nuclei. While we feel that both these explanations are possible, we consider them improbable. We feel that of all previously used techniques, autoradiography is the least susceptible to redistribution artefacts. The autoradiographic data presented here for the uterus suggest that there are bound nuclear receptors at 0–4 °C which appear in the cytoplasmic compartment on homogenisation¹⁵.

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Natural occurrence of 2-5A in interferon-treated EMC virus-infected L cells

B. R. G. Williams, R. R. Golgher*, R. E. Brown, C. S. Gilbert & I. M. Kerr

National Institute for Medical Research, Mill Hill, London NW7, UK

Until now the interferon-mediated 2'-5' adenine oligonucleotide inhibitors (2-5A) of cell-free protein synthesis have not been detected in intact cells. Here we report their natural occurrence in interferon-treated, EMC virus-infected mouse L cells in amounts consistent with the idea that they play a part in the inhibition of virus growth.

SOME years ago we demonstrated an interferon-mediated inhibition of cell-free protein synthesis in extracts prepared

from interferon-treated, virus-infected cells^{1,2}. Subsequently we reported that protein synthesis in cell-free systems from interferon-treated cells shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA)³. On the basis of these observations and the known effects of dsRNA in the intact cell and cell-free system⁴⁻⁶, we suggested³ that the production of viral dsRNA in response to infection (even with a DNA virus⁷) could bring about a general inhibition of protein synthesis in interferon-treated cells such as is indeed observed, for example, in interferon-treated, vaccinia virus-infected mouse L cells^{8,9}. The cell dies but little virus is produced: an effective way of limiting a natural infection. Alternatively, the triggering by dsRNA of a more transient or selective inhibition could result in the degradation of viral nucleic acid but allow the ultimate recovery of the cell. The enhanced sensitivity of cell-free protein

* Present address: Departamento de Microbiologia, Instituto de Ciencias Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

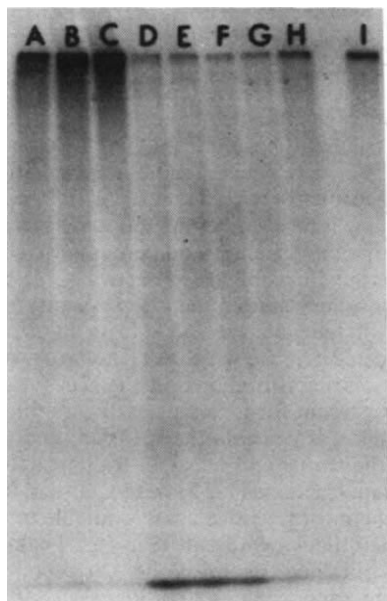


Fig. 1 Recovery of 2-5A from intact cells. 2-5A was assayed by its ability to activate a nuclease and cause the degradation of added ^{32}P -RNA in rabbit reticulocyte lysates²³. Autoradiographs of SDS polyacrylamide gel analyses of the ^{32}P -RNA are shown. Comparison of 2-5A activity isolated from control (A), EMC infected (B), interferon-treated (C) and interferon-treated, EMC virus-infected (D–H) cells at overall dilutions of 6 (A–D) and 18, 54, 162 and 486 (E–H) fold, respectively. I, control (no inhibitor). In typical assays of this type using standard preparations of 2-5A, nuclease activity is clearly detectable down to a final concentration of $\approx 2.4 \times 10^{-9}$ M (Fig. 3a, tracks 19–23). Mouse L cells (300 ml, 0.75×10^6 cells per ml) were treated for 17 h at 37°C with 400 reference units (20 effective units) per ml of partially purified (5×10^7 reference units per mg of protein) mouse interferon, infected with 20 PFU per cell of EMC virus and incubated for 4 h at 37°C . Control cells with or without interferon treatment or EMC infection were incubated in parallel. Cells were collected and washed, packed cells were lysed into an equal volume of 0.5% NP40 in 10 mM HEPES buffer, pH 7.5, 90 mM KCl, 1.5 mM magnesium acetate. The 100,000g supernatant fractions (500 μl) were heated to 95°C for 5 min, centrifuged to remove denatured protein and the supernatants fractionated on DEAE Sephadex²⁸. Fractions corresponding to 2-5A were eluted with 1 M NH_4HCO_3 , lyophilised extensively to remove bicarbonate and redissolved in 20 μl of H_2O before assay. In the nuclease assays here and in Figs 2 and 3, 30–300 ng (equivalent to 3,000–5,000 c.p.m. at a specific activity of 10^4 – 10^5 c.p.m. per μg) of ^{32}P -labelled polyadenylated mRNA (ref. 20) or 28S rRNA from Ehrlich ascites tumour cells were added per 12.5- μl assay. (Identical results were obtained throughout with the two types of RNA.)

synthesis to dsRNA showed the same interferon dose-response curve as did the antiviral effect in the intact cell, indicating that the two effects might indeed be closely related³. The basis for the effect of dsRNA was, therefore, investigated.

In the cell-free system two distinct mechanisms seem to be involved: (1) phosphorylation of the protein synthesis initiation factor eIF2 by an interferon and dsRNA mediated kinase; and (2) degradation of mRNA by a nuclease(s) activated by subnanomolar levels of an unusual oligonucleotide pppA2'p5'A2'p5'A (and related oligomers collectively referred to as 2-5A) synthesised by an interferon and dsRNA-mediated synthetase^{10–18}. In intact cells, 2-5A, when artificially introduced, has also been shown to be effective in the activation of a nuclease(s) and consequent inhibition of protein synthesis and virus growth^{19–21}. Both in the cell-free system and when introduced into intact cells 2-5A is unstable and in the absence of a 2-5A regenerating system the activation of the nuclease and inhibition of protein synthesis are transient^{18,20–22}. All of these results are consistent with a possible role for 2-5A in the inhibition of virus replication in the interferon-treated cell, but they do not provide direct evidence for it. More particularly, there has been no direct evidence for the natural occurrence of 2-5A, which has only been detected after synthesis in crude cell extracts or with the partially-purified synthetase. It has

remained possible, therefore, that it is an artefact of cell-free systems. We recently reported that 2-5A deliberately introduced into cells can be recovered from them²⁰. Using the same methods for the recovery of 2-5A here we report its natural occurrence in interferon-treated, encephalomyocarditis (EMC) virus-infected cells in amounts consistent with the idea that it plays a part in the inhibition of virus growth in these cells.

Presence of 2-5A in extracts from interferon-treated, EMC virus-infected L cells

Extracts were prepared from interferon-treated and control cells 4 h postinfection with EMC virus and centrifuged at 2,000 or 100,000g. The supernatant fractions were either precipitated with trichloroacetic acid (TCA, for the 2,000g supernatant) or heated to 95°C for 5 min (for the 100,000g), centrifuged to remove denatured protein and purified on DEAE Sephadex to remove the majority of contaminating mono- and oligonucleotides.

The fractions corresponding to the position at which 2-5A would have been expected to elute were pooled, lyophilised, redissolved in a small volume and assayed for 2-5A. Two types of assay were used: activation of a nuclease in the rabbit reticulocyte lysate and inhibition of protein synthesis in EMC RNA-programmed cell-free systems from Ehrlich ascites tumour (EAT) cells²³. In the first of these (Fig. 1A) ^{32}P -labelled mRNA was incubated in a rabbit reticulocyte lysate in the presence or absence of added test material. Whereas the addition of material from control cells plus or minus EMC infection or from interferon-treated cells was without significant effect (Fig. 1, A–C respectively), that from interferon-treated, EMC virus-infected cells showed evidence for activation of a nuclease(s) over a range of dilutions from 6 to 486-fold (Fig. 1, D–H). In accord with this, of these extracts, only that from interferon-treated, EMC virus-infected cells also inhibited protein synthesis at similar dilutions in the cell-free system. In an additional control experiment an extract from such cells, but not from a mixture of equal volumes of interferon-treated cells and EMC virus-infected cells, was similarly effective in activating a nuclease in the reticulocyte system. In this latter experiment the interferon-treated, EMC virus-infected cells and the mixture (interferon-treated cells plus EMC-infected cells) were each lysed with NP40 on ice and immediately precipitated with TCA. This was done to exclude the formal possibility that any 2-5A detected could have been synthesised during the processing of the extracts. (In fact, the extract prepared from the interferon-treated, EMC virus-infected cells in the latter more rapid fashion contained if anything slightly greater activity than did

Table 1 Trimer and tetramer triphosphate components of 2-5A recovered from HPLC of an extract from interferon-treated, EMC virus-infected L cells assayed by inhibition of cell-free protein synthesis

Dilution into the cell-free system	^{14}C -amino acid incorporation in the presence of dialysed and concentrated HPLC fractions (c.p.m. per 10 μl) [*]	
	25–29 (putative trimer, triphosphate)	35–39 (putative tetramer, triphosphate)
62.5	3,700	4,900
125	6,250	5,714
375	7,830	7,800
1,250	14,250	14,316
3,750	16,480	16,500

Pooled fractions 25–29 (750 μl) and 35–39 (750 μl) from the HPLC analysis of the interferon-treated, EMC virus-infected cell material shown in Fig. 2b were each dialysed against four changes of 100 volumes of H_2O at 4°C , lyophilised, redissolved in 20 μl of H_2O and assayed in an EMC RNA-programmed EAT cell-free system as described in Fig. 4. Standard trimer and tetramer preparations (750 μl of 1 μM in 0.2 M ammonium phosphate, pH 7.0) processed in parallel were each recovered in 80% yield. Non-phosphorylated A2'p5'A2'p5'A (1 μM) was added as 'carrier' before dialysis: parallel controls confirmed that it was without effect in the cell-free system.

^{*} Incorporation in the absence of added inhibitor was 17,100.

those prepared by the longer method involving the initial isolation of a 100,000g supernatant.) The material in the interferon-treated, infected cell extract responsible for the activation of the nuclease in the reticulocyte lysate was sensitive to digestion with bacterial alkaline phosphatase and snake venom phosphodiesterase, but resistant to digestion with T2 and micrococcal nucleases, as would be expected for 2-5A²⁴. Further evidence for the identity of this material as 2-5A came from its performance on HPLC in comparison with appropriate standards.

Characterisation of 2-5A by HPLC and comparison of the relative activities of the trimer and tetramer in the rabbit reticulocyte and EAT cell-free systems

2-5A synthesised with the synthetase from interferon-treated mouse L cells or rabbit reticulocytes consists of a mixture of oligonucleotides of the general formula $\text{ppp}(\text{A2'p})_n\text{A}$ with the trimer as the predominant species^{12,25}. The individual components, trimer, tetramer and pentamer ($n=2-4$, respectively) were isolated by chromatography on DEAE cellulose²⁵ and subjected to HPLC. In Fig. 2a the pattern obtained with a recombined mixture of these standard preparations is presented. Peaks 7 to 9 correspond to the trimer, tetramer and pentamer triphosphates, respectively. All preparations also contain smaller and variable amounts of the corresponding di- and monophosphates (peaks 4-6 and 1-3, respectively)²⁵. These are thought to represent breakdown products of the predominant triphosphates. (The terms trimer, tetramer and

pentamer will be used to denote preparations of this type consisting largely of the triphosphates, but also containing smaller amounts of the di- and monophosphates. The more complete nomenclature, for example trimer triphosphate, will be used to identify the individual species.) It has previously been shown that the trimer diphosphate (but not the monophosphate) has essentially the same specific biological activity as the corresponding triphosphate (ref. 25 and L. A. Ball, personal communication). As will be seen below, the same seems to be true for the corresponding tetramer and pentamer species. It is not known whether the diphosphates are active *per se* or are simply efficiently rephosphorylated in the assay systems used.

An analysis of the putative 2-5A from interferon-treated, EMC virus-infected cells (to which ATP and p_4A were added as optical density markers) is shown in Fig. 2b. Predictably, too little 2-5A was present for it to be clearly identified by optical density (although it is possible that peaks X and Y do indeed correspond to the trimer and tetramer triphosphates). Accordingly, appropriate fractions (1-53) were analysed for biological activity using the most sensitive assay available to us; nuclease activation in the reticulocyte lysate (Fig. 2c). Peaks of nuclease activity were obtained in positions corresponding to the tetramer di- and triphosphates (fractions 6-8 and 35-41) and the pentamer di- and triphosphates (fractions 11-13 and 45-47).

The apparent absence of trimer in the above preparation was curious but the explanation for it became clear from an analysis of the activity of appropriate standards. It seems that the trimer tri- and diphosphates (whether chemically or enzymically synthesised) are relatively poor activators of the nuclease and inhibitors of protein synthesis in rabbit reticulocyte lysates,

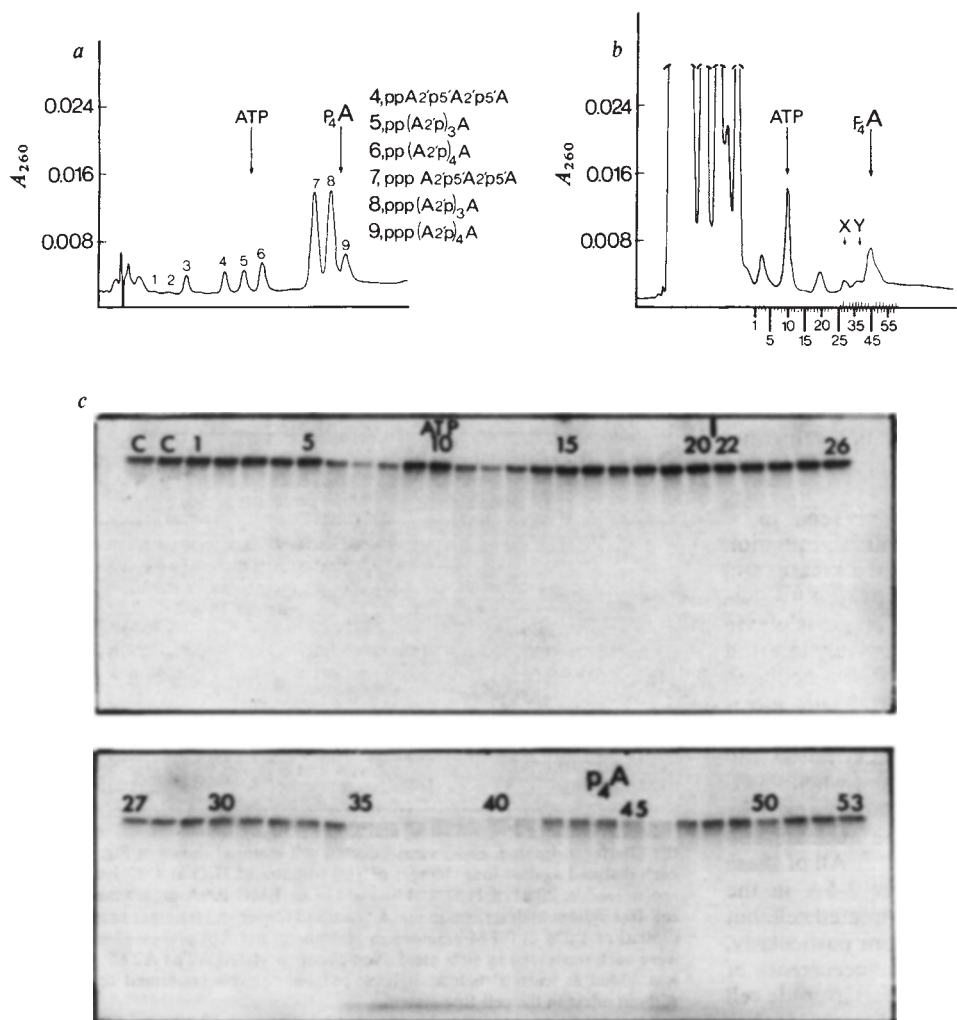
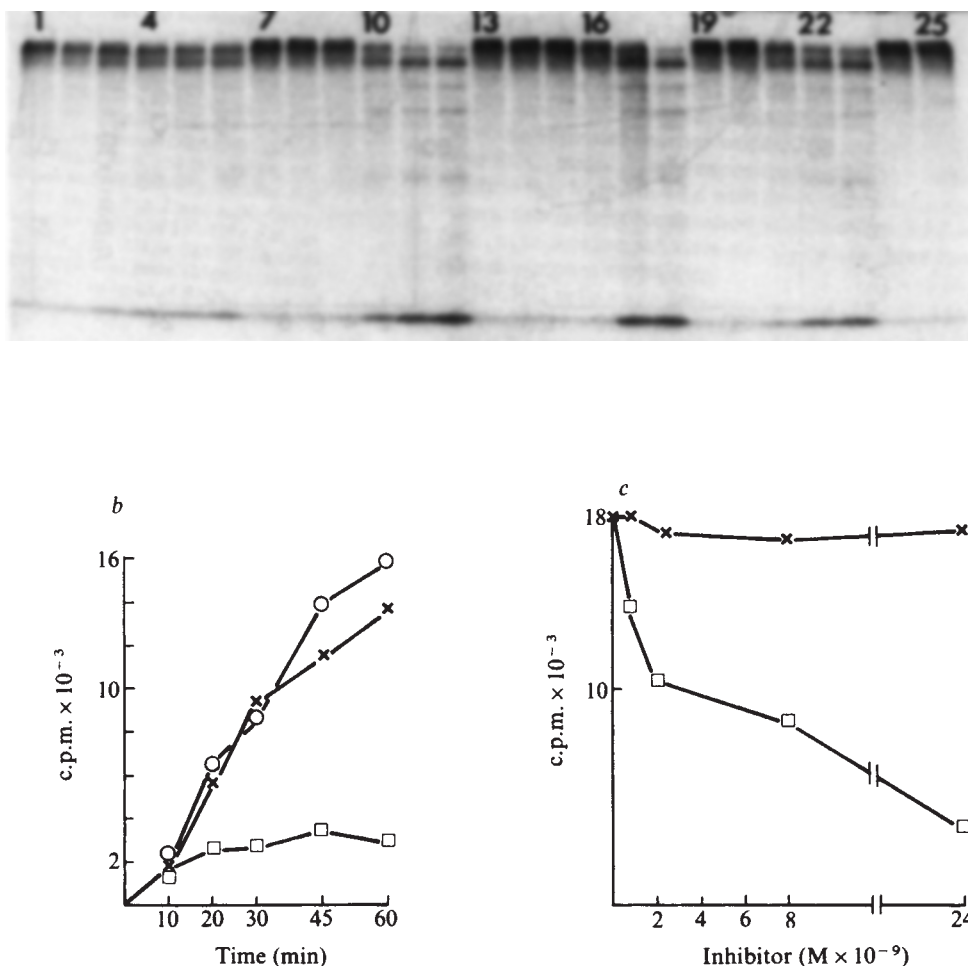


Fig. 2 HPLC analysis of 2-5A from interferon-treated, EMC virus-infected cells. *a*, A mixture of reticulocyte 2-5A standards containing the trimer, tetramer and pentamer triphosphates (peaks 7, 8 and 9 respectively) purified as described previously²⁵. Each contained lesser amounts of the corresponding di- (peaks 4-6) and mono- (peaks 1-3) phosphates. In a parallel run ATP and p_4A were included with the above components and the positions at which they eluted are indicated. *b*, 2-5A (3 μl) from interferon-treated, EMC virus-infected cells prepared as in Fig. 1 plus 5 μl each of 10 μM ATP and p_4A . *c*, Samples (2 μl of threefold dilutions) of each of the column fractions (1-20 and 22-53) (abscissa of *b*) were analysed for their ability to activate a nuclease and degrade added ^{32}P -RNA in the reticulocyte lysate system (12.5 μl , Fig. 1). The HPLC analysis was on a LiChrosorb 10 NH_2 column with a linear gradient of 40-200 mM ammonium phosphate, pH 7.2. 500 (1-25) and 250 (26-60) μl fractions were collected. (In our experience a number of amine columns from different manufacturers all gave excellent separations of the type shown in *a*. However, these columns apparently have a relatively short life in the conditions used allowing, on occasion, only as few as 30 to 50 runs to be made.)

Fig. 3 Comparison of the activities of the trimer, tetramer and pentamer components of reticulocyte 2-5A in the reticulocyte lysate system. *a*, Activation of a nuclease. Tracks 1–6: 2.4, 8, 24, 80, 240 and 800×10^{-9} M trimer. Tracks 7–12 and 13–18: 0.024, 0.08, 0.24, 0.8, 2.4 and 24×10^{-9} M tetramer and pentamer respectively. Tracks 19–23: 0.24, 0.8, 2.4, 8 and 24×10^{-9} M 2-5A (unfractionated). Tracks 24 and 25: no inhibitor controls. Assays were carried out as described in Fig. 1. *b*, *c*, Inhibition of 35 S-methionine incorporation in micrococcal nuclease treated rabbit reticulocyte lysate systems²⁹ programmed with EMC RNA²³. *b*, Time course of incorporation in the absence (○) and presence of 8×10^{-9} M trimer (×) and tetramer (□). *c*, Inhibitory activity of different concentrations (abscissa) of trimer (×) and tetramer (□). The ordinates give the 35 S-methionine incorporated (c.p.m. per 10-μl sample) into acid insoluble polypeptide with time of incubation at 30 °C (abscissa) in (*b*) and at 60 min in (*c*). The reticulocyte, trimer, tetramer and pentamer preparations were those analysed in Fig. 2*a*. The concentrations of these and of the unfractionated preparation of 2-5A are given in AMP equivalents. (1 ml of 1 mM inhibitor was taken to have an absorbance of 15 at 260 nm measured in a 1 cm pathlength cell.)



although apparently fully active in this respect in other systems (refs 12 and 25 and Fig. 4 below). The relative activities of the trimer, tetramer and pentamer in the activation of a nuclease in the reticulocyte lysate are compared in Fig. 3*a*. The tetramer and pentamer are both capable of clear-cut activation of a nuclease at final concentrations of 10^{-9} M. (The concentrations of the different species of 2-5A are given in AMP equivalents throughout.) Indeed, with fresh preparations of high specific activity RNA requiring the addition of smaller amounts of RNA, activation of nuclease can be detected with as little as 0.024×10^{-9} M of these components in this type of assay. Significantly, higher concentrations of the trimer are required ($>8 \times 10^{-9}$ M) and, more importantly, even with these higher concentrations the activation of the nuclease seems to be very much less efficient (Fig. 3*a*, tracks 1–6). In accord with this, 0.8 and 8×10^{-9} M tetramer (□) were sufficient to give detectable and substantial inhibitions respectively of amino acid incorporation in an EMC RNA-programmed reticulocyte lysate, whereas similar or greater concentrations of trimer (×) were without effect (Fig. 3*b*, *c*). In contrast the trimer (×) and tetramer (□) appeared almost equally effective in activating nuclease(s) (Fig. 4*a*, *b*) or inhibiting EMC RNA-programmed protein synthesis (Fig. 4*c*) in the EAT cell-free system.

The basis for the ineffectiveness of the trimer in the reticulocyte cell-free system is not known. It does not seem to be the result of a more rapid degradation of this component in this system and most probably, therefore, reflects a preference of the activatable nuclease for the tetramer or higher oligomers. Whatever the explanation, it is clear that the failure to detect trimer in the HPLC analysis of the 2-5A present in the inter-

feron-treated, EMC virus-infected cell extracts described in Fig. 2*b* could simply have reflected the choice of the reticulocyte as the assay system. This was shown to be the case by assaying appropriate fractions from the HPLC analysis (Fig. 2*b*) for the ability to activate a nuclease and inhibit protein synthesis in the EAT system. In a nuclease assay measuring conversion of 3 H-labelled VSV mRNA to acid solubles of the type shown in Fig. 4*a*, *b*, peaks were obtained in fractions 1 (corresponding to the trimer diphosphate), 7 (tetramer diphosphate), 26–28 (trimer triphosphate) and 36–38 (tetramer triphosphate), the results being consistent with there being, if anything, slightly more trimer than tetramer present (data not presented). The slightly lower sensitivity of the 14 C-labelled amino acid incorporation assay combined more importantly with its higher sensitivity to the 'non-specific' inhibitory effects of the salt gradient used in the elution from HPLC, made it impossible to detect convincingly even the triphosphorylated trimer and tetramer peaks by direct analysis of the column fractions in this type of assay. They were readily detected, however, in apparently identical amounts when appropriate column fractions were pooled, dialysed, concentrated and analysed in such systems (Table 1).

It can be concluded, therefore, that the triphosphorylated trimer, tetramer and pentamer components at least of 2-5A are present in interferon-treated mouse L cells 4 h postinfection with 20 plaque forming units (PFU) per cell of EMC virus. It is possible that smaller amounts of the diphosphates are also present in the intact cell, but it seems probable that these reflect partial breakdown products of the corresponding triphosphates. If higher oligomers are present they are not detectable with the

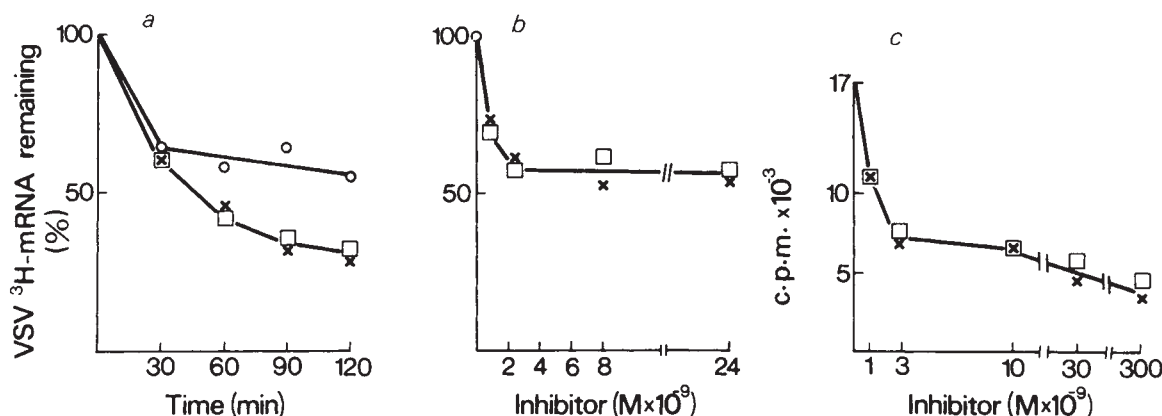


Fig. 4 Comparison of the activities of the trimer and tetramer components of reticulocyte 2-5A in EAT cell-free systems. *a, b*, Activation of a nuclease. Breakdown of VSV ³H-mRNA on incubation in an EAT cell-free system: *a*, alone (○) or with 8×10^{-9} M trimer (×) or tetramer (□). VSV ³H-mRNA (4×10^5 c.p.m. per μ g) was added to a final concentration of 2μ g ml⁻¹ and incubated in identical conditions to those previously described for L cell-free systems¹⁸ at 30 °C for the times indicated in (*a*) and 120 min in (*b*). The ordinates give the residual acid insoluble radioactivity as a percentage of the zero time value (2,600 c.p.m. per 4- μ l sample) in (*a*) and of the 120-min control (no inhibitor) value (3,500 c.p.m. per 10- μ l sample) in (*b*). (The 100% point in (*b*) corresponds to the 120 min control (○) point in (*a*)). *c*, Inhibition of amino acid incorporation by different concentrations (abscissa) of the trimer (×) and tetramer (□) in an EMC RNA-programmed EAT cell-free system prepared and incubated for 120 min at 30 °C as described for similar systems from mouse L cells^{18,30}. The ordinate gives the incorporation of a mixture of ¹⁴C-amino acids into acid insoluble polypeptide (c.p.m. per 10- μ l sample). The trimer and tetramer preparations were the same as those used in Figs 2a and 3. Their concentrations are given in AMP equivalents as described in Fig. 1.

procedures used here. The heterogeneity in the length of the 2-5A synthesised in cell extracts has been one of the odd aspects of the system for which as yet there is no explanation. This, perhaps more than anything else, emphasised the possibility that 2-5A, in the form isolated, might be no more than an intriguing artifact of cell-free systems. The discovery of similar heterogeneity here in the intact cell argues against this and, although providing no explanation for the phenomenon, suggests that it may have some significance.

Discussion

We have previously shown that 2-5A artificially introduced into cells is capable of activating a nuclease(s) and inhibiting protein synthesis and virus growth¹⁹⁻²¹. Here we have demonstrated the natural occurrence of 2-5A in amounts sufficient for it to be responsible for a similar sequence of events in interferon-treated, EMC virus-infected L cells. Assuming 50% recovery of the 2-5A throughout the isolation procedure (which seems reasonable from appropriate control experiments), the yields obtained in the four experiments completed have been consistent with concentrations of $20\text{--}200 \times 10^{-9}$ M 2-5A in the interferon-treated, EMC virus-infected cells from which the extracts were made. Preliminary evidence has also been obtained for an enhanced rate of breakdown of preformed RNA in these cells consistent with the activation of a nuclease (unpublished data). The relative importance of this mechanism, however, compared with any effect of the interferon and dsRNA mediated kinase, or alternative mechanism(s) not mediated through dsRNA, in this

and other interferon-treated, virus-infected cell systems remains to be established.

Here we have detected 2-5A only in the extracts from interferon-treated, virus-infected cells, whereas the wide distribution and variation in amount of the synthetase has raised the possibility that it may have a wider significance in the regulation of cell growth or development²⁶. The possible wider significance of the synthetase has been further emphasised by the recent demonstration by Ball that an enzyme which is almost certainly the 2-5A synthetase is capable (in cell extracts) of adding AMP in 2'-5' linkage to a variety of important metabolites such as NAD, ADP ribose and AppppA (ref. 27). On the other hand, were 2-5A to have been present in physiological concentrations of less than 10×10^{-9} M in any of the above cells we would not have detected it with the procedures used. Clearly, a more sensitive and convenient assay for 2-5A and a much more detailed analysis of this and alternative systems will be required before the full significance of either 2-5A or its synthetase can be assessed. Meanwhile, however, it is unlikely from the results presented here that 2-5A is a mere artefact of the cell-free system and it is reasonable to assume that it plays some part in the inhibition of virus growth in the interferon-treated, EMC virus-infected cell.

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letters

Observations of a flaring X-ray pulsar in Dorado

E. P. Mazets, S. V. Golenetskii, V. N. Il'inskii,
R. L. Aptekar' & Yu. A. Guryan

A. F. Ioffe Physico-Technical Institute, Leningrad, 194021, USSR

The γ -ray burst detector Konus¹, on the Venera 11 and Venera 12 spacecraft, detected on 5 and 6 March, 1979 two bursts of hard X rays originating from the same source. These events are quite unusual and of considerable interest. The burst of 5 March was very intense, particularly in the initial phase. This event was also observed by several other spacecraft². The second burst on 6 March was considerably weaker. The observations reported here permitted us to obtain a detailed time structure of the bursts, to measure their energy spectra and to locate the source on the celestial sphere.

Figure 1 displays the time structure of the initial phase of the 5 March burst recorded on Venera 11 in the energy range 50–150 keV with a time resolution of 1/64 s. Note that all results obtained on Venera 11 and Venera 12 coincide completely. The burst has a very sharp onset. The initial, narrow structureless pulse is characterised by a very short rise time, ~ 15 ms, and a longer decay, ~ 150 ms. The peak count rate in the 50 cm² sensor attains 4×10^5 c.p.s. This means that during a short time (~ 0.1 s), the flux of hard X rays exceeded the diffuse cosmic background by a factor of 10^4 . The time structure of the burst measured with resolutions of 1/4 and 1 s is shown in Fig. 2a, b. An analysis of these data clearly indicates that the radiation observed is that of an X-ray pulsar with a period 8.1 ± 0.1 s (ref. 3). The pulsed radiation is seen to exhibit a train of stronger pulses and another train of interpulses with a relative phase of 0.5. The level of intensity in the deep minima between the pulses apparently reflects a slowly varying component of radiation.

The most remarkable fact here is that the onset of radiation is almost instantaneous with a subsequent fast decay. The standard length of measurements (66 s) was found to be insufficient for this event; however, measurements carried out 6 min after the beginning of the burst revealed that by this time the intensity of radiation had decreased to background level.

Eight consecutive measurements of the energy spectra were carried out, each of 4 s duration. Only the first spectrum measured in the initial burst phase differs in shape from the others. Therefore, Fig. 3 presents the energy spectrum of the burst for the first time interval, 0–4 s (histogram a), and a spectrum averaged over the interval 4–32 s (histogram b). The energy spectra of this event differ from those of most of the γ -bursts⁴. The shape of the pulsed radiation spectrum is more similar to those of the spectra of continuously emitting X-ray sources, such as Cyg X-1, than to the substantially harder spectra of typical γ bursts. It may be approximated by the relationship $E^{-1} \exp(-E/kT)$, with $kT = 30$ keV.

The spectrum of the initial burst phase has a harder tail, which is possibly associated with radiation of only the initial pulse. The spectral feature in the region 400–500 keV is a remarkable characteristic of this spectrum. The total identity of the spectra obtained on Venera 11 and Venera 12 completely eliminates the

possibility that this feature is a result of random fluctuations. A more detailed analysis of the spectrum shows that the observed feature may be associated with the presence of a broad line at 430 keV with a FWHM of 30–40%. It is difficult to suggest an unambiguous explanation of the nature of this radiation component. Note, however, that 430 keV corresponds to the energy of the 511-keV annihilation line which has undergone a redshift of $(1 - 2GM/c^2R)^{1/2}$ in the gravitational field of a compact object with $M = 1M_\odot$ and $R = 10^6$ cm.

The average pulsed radiation flux with $E_\gamma > 30$ keV is 1×10^{-5} erg cm⁻² s⁻¹. At the peak of the initial pulse the flux is 1.5×10^{-3} erg cm⁻² s⁻¹. Evaluation of the flux in the line with $E_\gamma \approx 430$ keV yields 3×10^{-6} erg cm⁻² s⁻¹, or, assuming the line radiation to be emitted only in the initial pulse, 5×10^{-5} erg cm⁻² s⁻¹.

The location of this flaring X-ray pulsar, designated earlier FXP 0520-66 (ref. 3), is shown in Fig. 4. The readings of the Konus sensor unit with anisotropic angular sensitivity yield a fairly large error box with sides of about 3°. Intersection of this region with a narrow annulus obtained from the time-of-arrival difference between Venera 11 and Venera 12 gives the final box as a narrow annular band 4' wide. Based on the latest spacecraft trajectory data, the position of this annulus on the celestial sphere is defined by the angular radius $68.528 \pm 0.030^\circ$ with the centre at $\alpha = 44.427^\circ$, $\delta = -2.747^\circ$ (1950.0). This position is in good agreement with other data. The arc of radius 68.528° passes 1.7 arc min to the south-east of the centre of the error box for this source given in ref. 2— $\alpha = 5$ h 25.92 min, $\delta = -66.122^\circ$.

As mentioned above, the observed source was found to be recurrent. On the next day, 6 March, the Konus detector on both spacecraft recorded, after the same time interval $\Delta t = 14$ h 25 min 46.15 s a short burst ~ 1.5 s long with the same soft

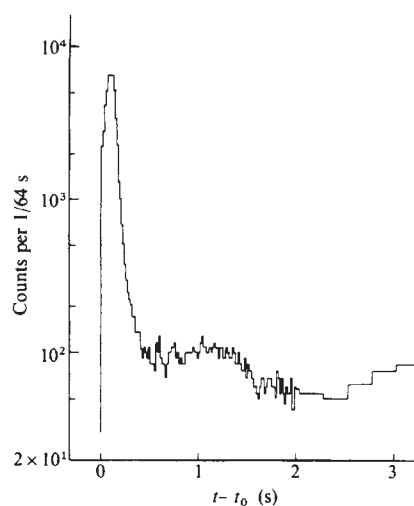


Fig. 1 Initial stage of the 5 March event as recorded on Venera 11. The data of Venera 12 are identical. Energy range covered 50–150 keV. Time resolution, 1/64 s. Burst onset time t_0 . Venera 11: 15 h 51 min 39 s, 145 UT. Venera 12: 15 h 51 min 44 s, 350 UT.

spectrum (Fig. 3, histogram *c*). Its intensity, however, was 100 times lower. Its time structure is shown in Fig. 2*c, d*, with results of source location determination presented in Fig. 4. For the 6 March event, the process responsible for the emission in the source was evidently weaker and longer than in the case of the 5 March burst. We can assume that the decaying pulsed radiation could have been accordingly weaker and, hence, not observable against the background present.

The most plausible model for the FXP0520–66 source seems to be a binary system containing a neutron star. The separation between the components should be sufficiently large to reduce the accretion rate to the level where there would be no observable radiation in the steady state. The neutron star should possess a strong magnetic field; indeed, the presence of two pulse trains in the burst implies that during accretion the plasma moves along the field lines with the emitting regions located near the magnetic poles. The origin of the non-stationary accretion resulting in a burst remains unclear. The characteristic rise time to almost full peak luminosity is small, less than 15 ms, implying that processes associated with the neutron star proper, such as the development of instabilities in the magnetosphere, should play the part of the trigger mechanism.

The observed source projects on to the Large Magellanic Cloud. Moreover, it lies close in position to the supernova remnant N49 in the Large Magellanic Cloud². However, its energetics suggest that it is not only located in our Galaxy, but even comparatively close to the Sun. Indeed, if the source were at 55 kpc the luminosity of isotropic radiation in the initial impulse would be $\sim 5 \times 10^{44} \text{ erg s}^{-1}$, and that at the pulsating stage, $\sim 3.6 \times 10^{42} \text{ erg s}^{-1}$. Accordingly, the energy released in the initial impulse would be $\sim 1.2 \times 10^{44} \text{ erg}$, that at pulsating stage (lasting $\sim 100 \text{ s}$) $\sim 3.6 \times 10^{44} \text{ erg}$, and in line radiation, $\sim 10^{42} \text{ erg}$. The total energy in the event would then be $> 4.6 \times$

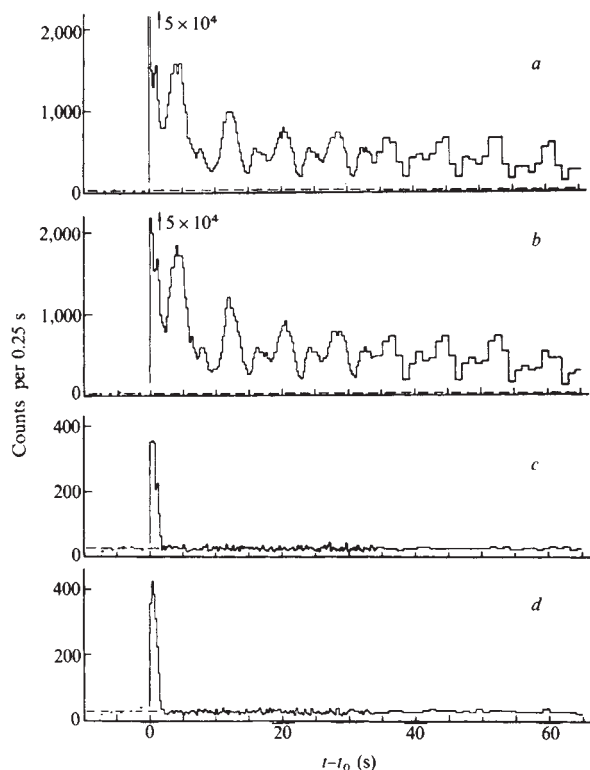


Fig. 2 Time structure of the bursts with resolution 1/4 and 1 s in the energy range 50–150 keV. *a*, 5 March, Venera 12; *b*, 5 March, Venera 11; *c*, 6 March, Venera 12: $t_0 = 6 \text{ h } 17 \text{ min } 30 \text{ s}$, 455 UT. *d*, 6 March, Venera 11: $t_0 = 6 \text{ h } 17 \text{ min } 25 \text{ s}$, 200 UT. Dashed line indicates background count rate. Points before t_0 show previous history of the bursts.

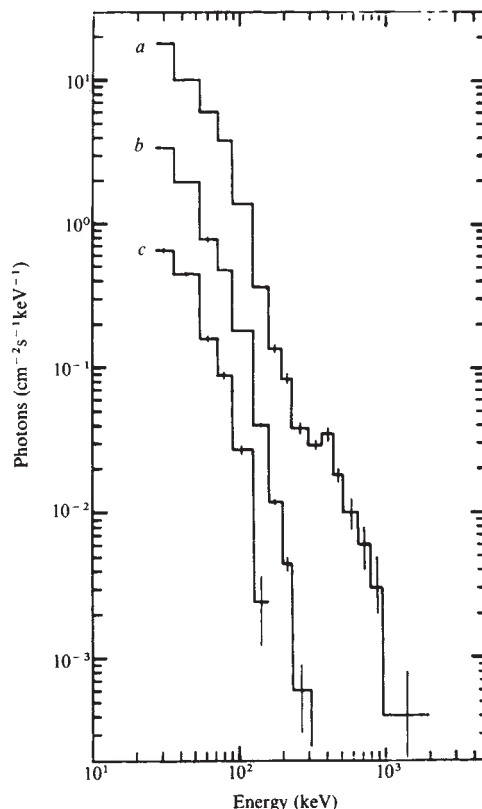


Fig. 3 Burst energy spectra. *a*, 5 March, spectrum of initial stage for the first 4 s; *b*, 5 March, averaged spectrum of pulsed radiation; *c*, spectrum of 6 March burst. Data from Venera 11 and Venera 12 are identical.

10^{44} erg . The shape of pulsations (Fig. 2) shows that the angular pattern of emission cannot be very narrow. Taking into account possible directivity of emission could not reduce these estimates by more than a factor of 10. Such large figures for the total energy and luminosity apparently rule out the possibility that this source is located in the Large Magellanic Cloud. On the other hand, if its average luminosity in the pulsating phase is close to that of X-ray sources in binaries (10^{37} – $10^{38} \text{ erg s}^{-1}$), an estimated distance to it is 100–300 pc.

Consider some implications of the assumption on the galactic nature of this unusual X-ray pulsar. In its characteristics, mainly in the time scale of its variability and the presence of an impulsive initial stage, the FXP0520–66 source differs

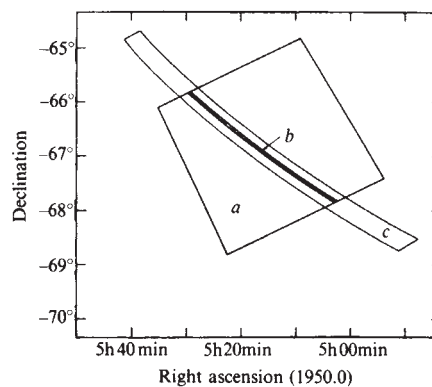


Fig. 4 Location of FXP 0520–66 source. Error box: *a*, for 5 March event, determined from detector anisotropic angular response; *b*, for 5 March event, obtained from detector angular response and time-of-arrival difference; *c*, same for 6 March event.

markedly from the known transient sources⁵. It is unlikely that this source is the only one in the Galaxy. Other such sources probably exist and should be observable. However, the distances to them should be, on average, much larger, about a few kpc. Therefore, the observable radiation flux from such sources should be small. This implies that flaring X-ray pulsars could be detected primarily in their initial impulsive phase as short bursts of hard X-ray radiation.

Do the abovementioned differences in the spectral characteristics indicate that a binary cannot be the origin of γ -ray bursts? Apparently, not. Indeed, the revealed concentration of the γ -burst source locations in the direction of the galactic centre implies that distances to them are large and should be, on average, a few kpc (ref. 4). The corresponding estimates of the average energy released in γ -ray burst sources, 10^{40} – 10^{41} erg, yield a value of 10^{39} – 10^{40} erg s⁻¹ for the average luminosity. These values exceed by at least 2 orders of magnitude the luminosity of known binary X-ray sources, as well as that of the FXP0520–66 source in its pulsating stage. Therefore, it is natural to suggest that the hardness and the shape of the radiation spectrum are substantially dependent on accretion rate, and it is the very high power of non-stationary accretion that determines the typically hard energy spectra of the γ -ray bursts that approach the power law in shape. The presence in the 5 March event of a hard component in the energy spectrum of the powerful initial impulse strongly supports this assumption.

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X-ray observations of the 5 March 1979 γ -burst field

David J. Helfand & Knox S. Long

Columbia Astrophysics Laboratory, Departments of Astronomy and Physics, Columbia University, 538 West 120th Street, New York, New York 10027

On 5 March 1979, an extremely intense burst of hard X rays and γ rays was recorded by the nine interplanetary spacecraft of the burst sensor network and localised by time-of-flight determinations to a position coincident with the supernova remnant N49 in the Large Magellanic Cloud^{1–3}. Several times, both before and after the γ -ray event, we observed this region of the sky with the soft X-ray imaging instruments aboard the Einstein Observatory. Coupled with optical plate material, the soft X-ray data are used here to place severe constraints on models for the origin of this remarkable transient phenomenon.

The γ -ray observations are reported in the preceding letter². Briefly, the salient features of the event include an extremely high peak flux (1.5×10^{-3} erg (cm² s)⁻¹ above 30 keV), a rapid rise time (≤ 0.25 ms, ref. 1), a decline to a flux level 10^{-2} times that of the peak within a second, followed by a slow (~ 100 s) decay characterised by 8.1-s pulsations, a soft γ -ray spectrum which during the initial burst shows evidence of a line feature near 430 keV, and a second much weaker burst 14 h later. The morphology, period and spectrum of the pulsed component are reminiscent of pulsating binary X-ray sources, such as Her X-1 and Cen X-3, which are thought to contain magnetised rotating neutron stars and to be powered by accretion from a normal companion star. The case for the presence of a neutron star in the system responsible for the γ -ray event is strengthened if one

interprets the 430 keV emission line as redshifted electron-positron annihilation radiation. The implied gravitational field is just that found due to a $1M_{\odot}$ object with a radius of 10 km. The positional coincidence of the γ -ray event and a supernova remnant (SNR) might be cited as further evidence that a neutron star was involved; however, the luminosities implied by the association of this event with the Large Magellanic Cloud are quite large: $L_{\gamma} \sim 5 \times 10^{44}$ erg s⁻¹ at the burst peak and $L_{\gamma} \sim 4 \times 10^{42}$ erg s⁻¹ during the pulsing phase. In what follows, the X-ray data are presented and then used to set limits on possible interpretations for the origin of the event.

The X-ray observations were carried out as part of a complete soft X-ray survey of the Large Magellanic Cloud that has been underway since the beginning of the year⁴. The instrumentation of the Einstein Observatory has been described elsewhere⁵. With the imaging proportional counter (IPC) in the focal plane of the telescope, a $1^{\circ} \times 1^{\circ}$ field of view is imaged with a spatial resolution of 1.5' (FWHM), while the spatial resolution obtainable with the high resolution imager (HRI) over a $25' \times 25'$ field is 4". The initial IPC pointing, which occurred 8 days before the burst, revealed the presence of two bright sources of soft X-ray emission centred on N49 and (N49), emission nebulae in the Large Magellanic Cloud previously identified as SNRs⁶. With the moderate resolution of the IPC, N49 was unresolved; its companion 6' to the north-west showed some evidence of extended structure. The results of the subsequent HRI observations are shown in Fig. 1. The source associated with (N49) is seen to be a uniform, symmetric emission region with a diameter of ~ 40 pc. By contrast, N49 is ~ 25 pc in diameter with a strong enhancement in the south-east quadrant and bright knots of emission similar to those observed by Dopita and Mathewson⁷ and D. Clark (personal communication) in recent maps of coronal [Fe XIV] emission. A detailed discussion of the X-ray structure of SNR in the Large Magellanic Cloud will be given elsewhere. Here, we concentrate on those aspects of the data relevant to the 5 March event.

Our first important constraint on models for the γ -ray burst derives from the constancy of the flux from the N49 region in the two IPC observations carried out 0.72×10^6 s before and 3.29×10^6 s after the γ -ray event. We can quantify this constancy, independent of instrumental or background effects, by taking the ratio of the flux in the region of N49 to that from (N49). These ratios in the two IPC images are 2.167 ± 0.09 and 2.185 ± 0.09 , corresponding to a limit on the change in flux from N49 of $< 0.8\%_{-0.8\%}^{+2.9\%}$. Assuming a power law spectral index of -2 and a low energy cutoff of 0.5 keV, this corresponds to a 3σ upper limit on the change in flux of 2.2×10^{-12} erg (cm² s)⁻¹.

The improved spatial resolution of the HRI permits us to derive a second result—namely, a limit on the strength of any point source within the instrument's $25' \times 25'$ field of view. This limit is obtained by summing the number of counts detected in $8'' \times 8''$ regions of the map and then subtracting a local background counting rate determined from the eight adjacent regions. The limits thus obtained depend on the mean surface brightness of the area being searched. For the region of extended emission within N49, the 3σ upper limit is 9.9×10^{-3} c.p.s., whereas for the area outside N49 but still near the γ -ray error box, the limit is $\sim 10^{-3}$ c.p.s. These point source limits correspond respectively to flux limits of 2.1×10^{-12} erg (cm² s)⁻¹ and 2.2×10^{-13} erg (cm² s)⁻¹ (0.5–4.5 keV). The greater of these two values is therefore essentially the same as the limit to any change in flux derived from the IPC.

These X-ray limits imply that, independent of distance, the steady-state (or remnant) luminosity from the system which produced the γ -ray event is less than 10^{-9} that observed above 30 keV in the burst. This translates to a luminosity limit of $2 \times 10^{30} d_{100}^2$ erg s⁻¹, where d_{100} is the distance to the source in units of 100 pc. At the Large Magellanic Cloud, the limit on L_x is $< 4 \times 10^{35}$ erg s⁻¹, two orders of magnitude smaller than that of a typical pulsating binary X-ray source.

In Table 1, we present these limits along with the γ -ray luminosities and energies and a variety of model-dependent

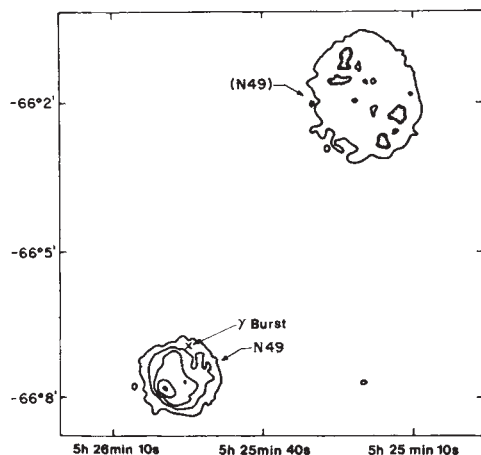


Fig. 1 Surface brightness contour map of the region containing N49 and (N49) as observed with the HRI. The map has been smoothed by convolving the raw data with a gaussian with a σ of $3''$. The contour levels correspond to 0.025, 0.10, 0.20, 0.40 and 0.62 counts $[(1' \times 1') s]^{-1}$. The lowest contour ($\sim 3\sigma$ above the mean background level) corresponds to an equivalent flux of approximately $10^{-6} \text{ erg (cm}^2 \text{ s keV)}^{-1}$ in the energy range 0.5–4.5 keV. The position of the γ -ray burst (ref. 1) is shown; preliminary errors on this position are $\sim 1.25'$ in right ascension and $1.5'$ in declination.

constraints implied by the observations. The sample distances are for a very nearby object (1 pc; see below), 200 pc suggested by Mazets *et al.*², 2 kpc corresponding to the limit for a galactic object in this direction ($|z| < 1 \text{ kpc}$), and 55 kpc, the distance of the Large Magellanic Cloud⁸. The neutron star temperature limits T_{ns} have been derived by convolving the radiation expected from an isotropically emitting black body of radius 10 km with the transmission characteristics of the interstellar medium (see ref. 9 for details). The masses of material involved in burst I on 5 March and burst II the following day, m_I and m_{II} , have been derived from the expression

$$m = \frac{E_\gamma}{c^2} \frac{1}{\eta_b} \quad (1)$$

The results scale linearly with η_b , the efficiency with which the rest mass energy is converted to γ rays above 30 keV; as an example we have adopted a value of $\eta_b = 0.03$ appropriate for the conversion of hydrogen to helium in a thermonuclear flash. An interim mass accretion rate $\dot{m}_{I-II}$ is implied if the mass

involved in burst II was accumulated between the events, while an upper limit to the steady-state accretion rate $\dot{m}_x$ can be derived from the X-ray observations:

$$\dot{m}_x < \frac{L_x}{c^2} \frac{1}{\eta_x} \quad (2)$$

where the standard value for the conversion of accreted material to X rays in a typical X-ray binary system of $\eta_x = 0.1$ has been used. These two values imply that the accretion rate is highly variable, since

$$\frac{\dot{m}_{I-II}}{\dot{m}_x} > 200 \frac{\eta_x}{\eta_b} \quad (3)$$

Finally, the spectral type of any normal star associated with the system responsible for the burst is constrained by the fact that there is no star brighter than $m_B \sim 14.5$ on the ESO sky survey blue plate near or within the γ -ray error box; this observation rules out all but late-type, main sequence companions for the galactic distances and supergiant companions in the Large Magellanic Cloud.

Having tabulated these results, we consider the probable nature and distance of the system which produced the γ -ray event.

The remarkable positional coincidence of the γ -ray burst and N49 shown in Fig. 1 is the strongest argument for locating the system responsible in the Large Magellanic Cloud. Mazets *et al.*² reject this hypothesis because of the luminosities it implies. However, their implicit application of Eddington-limited accretion to the luminosity during the pulsation phase is clearly not required for such nonsteady flow. For example, the observed pulsed luminosity of $4 \times 10^{42} \text{ erg s}^{-1}$ (for a 55-kpc distance) is consistent with the thermal emission expected from a hot polar region with the observed temperature of 30 keV covering 10% of a neutron star surface. While current opinion seems to favour a galactic origin for γ -ray bursts¹⁰, their spatial distribution is isotropic and consistent with an extragalactic origin. The fact that this burst was extremely bright may simply reflect its location in our nearest extragalactic neighbour, the Large Magellanic Cloud. However, this argument encounters severe difficulties if we assume that, despite some spectral and temporal differences, this γ -ray event is a manifestation of the same underlying phenomenon which produces all γ -ray bursts. The 5 March event was a factor of 10^2 – 10^3 more intense than other observed bursts. For an intrinsic spread in burst luminosities of an order of magnitude, this implies that the event was between 3 and 100 times closer than other bursts. An origin in the Large Magellanic Cloud, then, implies typical distances of 0.2 to 5 Mpc

Table 1 γ -Ray burst parameters

Parameter	Distance (pc)			
	1	200	2,000	55,000
$L_{\gamma I}$ (peak) (erg s^{-1})	2×10^{35}	7×10^{39}	7×10^{41}	5×10^{44}
$L_{\gamma I}$ (pulsed) (erg s^{-1})	1×10^{33}	5×10^{37}	5×10^{39}	4×10^{42}
$E_{\gamma I}$ (erg)	1.5×10^{35}	6×10^{39}	6×10^{41}	4.5×10^{44}
$E_{\gamma II}$ (erg)	1.5×10^{33}	6×10^{37}	6×10^{39}	4.6×10^{42}
L_x and ΔL_x (erg s^{-1})	$< 1 \times 10^{26}$	$< 5 \times 10^{30}$	$< 5 \times 10^{32}$	$< 3.5 \times 10^{35}$
T_{ns}^* (K)	$< 1 \times 10^5$	$< 3.5 \times 10^5$	$< 9 \times 10^5$	$< 3.5 \times 10^6$
m_I (g)	6×10^{15}	2×10^{20}	2×10^{22}	1.5×10^{25}
m_{II} (g)	6×10^{13}	2×10^{18}	2×10^{20}	1.5×10^{23}
$\dot{m}_{I-II}$ (g s^{-1})	1×10^9	4×10^{13}	4×10^{15}	3×10^{18}
$\dot{m}_x$ (g s^{-1})	$< 1.5 \times 10^6$	$< 6 \times 10^{10}$	$< 6 \times 10^{12}$	$< 4 \times 10^{15}$
Companions allowed	None	Dwarfs and subdwarfs later than K5; white dwarfs	Dwarfs and subdwarfs later than FO; white dwarfs	All but supergiants

* Values of 1 cm^{-3} for the galactic interstellar hydrogen density and 10^{21} cm^{-2} for the total column density to the Large Magellanic Cloud have been adopted.

which represent a volume of space containing too few galaxies to produce an isotropic burst distribution. Thus, starting from the Large Magellanic Cloud hypothesis, we are forced to conclude that either this burst represents a distinct class of events as suggested by Cline *et al.*³ with a different underlying mechanism, or that it was more than 10 times weaker than a typical burst implying mean intrinsic luminosities for this phenomenon of $>10^{46}$ erg s⁻¹.

The same distance scaling factors (3–100 times closer) also apply to a galactic hypothesis. For a scale height of 1 kpc which is consistent with the observed burst distribution¹⁰, this event must have been located between 10 and 300 pc from the Earth. Models for the origin of such a nearby event are constrained by the various limits presented in Table 1. Neutron star temperature limits of a few hundred thousand degrees are only marginally consistent with those expected from undisturbed, isolated objects⁹. In addition, the various accretion rates coupled with the limits on an optical companion can be used to constrain the parameters of models containing binary systems.

By using the observation of the narrow 430-keV emission feature observed in the burst spectrum, we can derive another estimate of the source distance. If this feature is real, an upper limit to the distance of the neutron star may be obtained if we assume that the continuum emission arises from electron bremsstrahlung and that the emission region (assumed for simplicity to be spherical and of uniform density) is the same for both the line and the continuum. For a narrow line feature to exist, the electron scattering depth must not greatly exceed unity; that is,

$$\sigma n_e r = \tau_{es} \leq 1 \quad (4)$$

where σ is the electron scattering cross-section at 430 keV, n_e is the ambient electron density and r is the radius of the emission region. The distance of the event determined from the continuum radiation is given by

$$d = 3^{-1/2} F^{-1/2} \varepsilon^{1/2} n_e r^{3/2} \quad (5)$$

where F is the continuum flux and ε ($\sim 1.64 \times 10^{-27} T^{1/2}$ erg cm⁻³) is the volume emissivity of a gas with unit density. Thus, in units appropriate if the positron line were present only during the initial fast part of the burst and assuming that the light travel time of this portion of the event sets a limit on the size of the emitting region, we have

$$d \leq 2.3 \times 10^{18} \left(\frac{1.5 \times 10^{-3} \text{ erg (cm}^2 \text{ s)}^{-1}}{F} \right)^{1/2} \times \left(\frac{T}{30 \text{ keV}} \right)^{1/4} \tau_{es} \left(\frac{t}{10^{-3} \text{ s}} \right)^{1/2} \text{ cm} \quad (6)$$

which is <1 pc! Unfortunately, the time resolution of the spectra presented by Mazets *et al.*² is 4 s. If values for the continuum flux are determined over this interval and the diameter of the emitting volume is assumed to be 4 light s, the upper limit is still less than 100 pc. The closest distances are probably excluded, in that X rays from interstellar accretion onto an isolated neutron star would violate our point source flux limits. We note that for $d \leq 10$ pc, however, such an isolated star can accumulate sufficient interstellar material to satisfy the entire γ -ray burst energy requirements in <10 years.

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Solar structure from global studies of the 5-minute oscillation

A. Claverie, G. R. Isaak, C. P. McLeod
& H. B. van der Raay

Department of Physics, University of Birmingham, Birmingham, UK

T. Roca Cortes

Instituto de Astrofísica de Canarias, La Laguna, Tenerife

High sensitivity Doppler spectroscopy of integral sunlight in the 769.9 nm line of neutral potassium reveals several equally spaced lines of $Q > 1,000$ centred on the well known period of 5 min. These lines are interpreted as low l , high n overtones of the entire Sun and, if viewed in terms of current solar models, appear to imply a low heavy element abundance and a consequent low solar neutrino flux. Previous workers^{1–3} have used spatial resolution to study the 5-min oscillation of the solar photosphere and have shown that power is concentrated along narrow ridges in the k, ω diagram. These oscillations are mainly low n , high l value acoustic modes of the entire Sun. In contrast to this, the present work (for a summary see ref. 4) is aimed at an overall view of the solar surface and consequently is biased to a study of low l value oscillations. These modes penetrate deeply⁵ and thus probe the structure as well as the interior angular velocity of the Sun⁶.

Doppler shift measurements of integral solar light show several discrete lines of amplitude 0.1–0.3 m s⁻¹ within the main peak of the power spectrum of the 5-min oscillation. These lines are found to have, on average, a uniform spacing of 67.8 μ Hz.

If the lines are interpreted as being due to low l value oscillations of the entire Sun, then the observed spacing taken in the context of current solar models^{7,8} implies a mean heavy element abundance of $Z \sim 0.004$ and a consequent neutrino flux of ~ 2.3 SNU, within two standard deviations of the experimental result⁹.

Line of sight velocity measurements of the whole solar disk were made using optical resonance spectroscopy, a technique

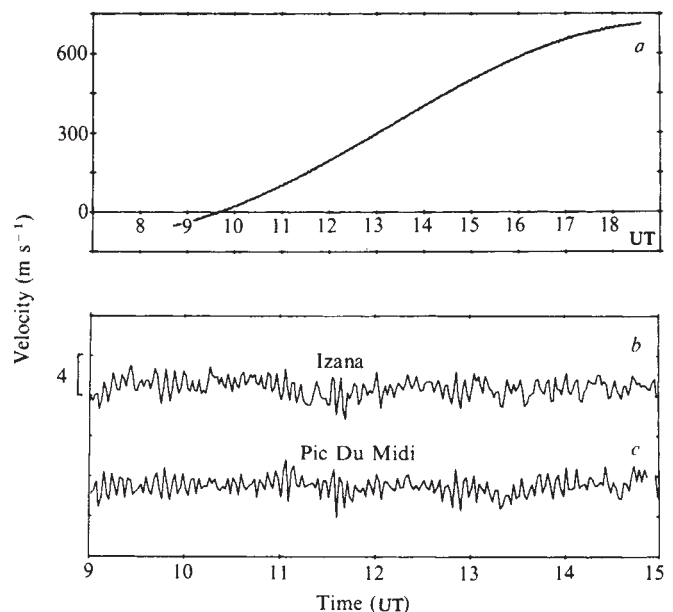


Fig. 1 Mean line of sight velocity observed on 6 August 1978 at Izana (a). Comparison of residuals obtained simultaneously at Izana (b) and Pic du Midi (c).

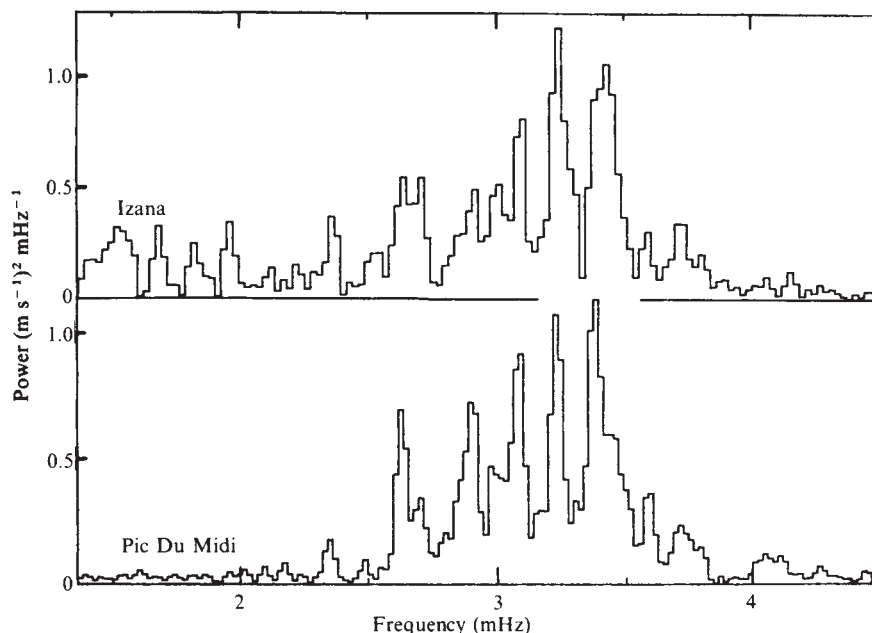


Fig. 2 Power spectra of the residuals obtained from data taken on 6 August at Izana and Pic du Midi.

which has been described in detail previously¹⁰. Briefly, the velocity of the solar photosphere with respect to the laboratory is measured by comparing the position of the Fraunhofer absorption line of neutral potassium at 769.9 nm with that of the same line in the laboratory. Observations were made during 1976, 1977 and 1978 at Izana, Tenerife and in 1978 simultaneously at Pic du Midi in the Pyrenees. The two sites are $\sim 2,300$ km apart and are expected to be meteorologically uncorrelated, thus allowing solar effects to be distinguished from atmospheric or instrumental perturbations. In the 1977 and 1978 seasons the spectrometer incorporated substantial improvements with respect to guidance, optical components, thermal stability and statistical accuracy, resulting in the collection of data of improved quality.

The Sun was observed for about 8 h on each of 33 days in 1976 (a total of 269 h) and 35 days in 1977 (total 280 h). These data as well as those from 7 of the days obtained in 1978 have been analysed. The data for any one day consist of a mean line of sight velocity determined every 42 s (100 s on Pic du Midi) for the whole observing day (Fig. 1a). Residuals corresponding to velocity amplitudes of less than 3 ms^{-1} (Fig. 1b) show up after subtraction of the observer's velocity relative to the centroid of the Sun due to the spin and orbital velocities of the Earth. A small allowance for residual curvature effects has been made.

Residuals of data obtained simultaneously at the other observing site are shown for comparison in Fig. 1c. To compare these data directly, those from Izana have been smoothed by a moving mean over three points (126 s) and then summed over two points (84 s) to simulate the 100-s integration time of the Pic du Midi data. The correlation of these two data strings clearly demonstrates that the observed signals can only be attributed to a solar origin.

These residuals form a time series which may be processed using standard power spectrum techniques¹¹. The power spectra obtained for the data of Fig. 1b and c are shown in Fig. 2. The agreement of these two spectra, obtained simultaneously at two sites separated by 2,300 km, clearly reinforces the statement that the observed oscillations are of solar origin. A series of well defined peaks can also be seen.

To investigate these peaks further the power spectra for individual days were averaged for the 1976, 1977 and 1978 data. These spectra are shown in Fig. 3. The structure is clearly present and an apparent constancy in the spacing of these peaks can be seen. To verify that this spacing is not an artefact of the analysis, different lengths of data strings were used but this had no effect on the observed spacing.

The constancy of the peak spacing may be demonstrated in two ways. First, the observed peaks in the power spectra are numbered sequentially and plots of the order of the peak against the observed frequency are shown in Fig. 4a for the 3 yr of data. Where the line spacing clearly suggests that a particular line is absent in the experimental data, the order of the line has been

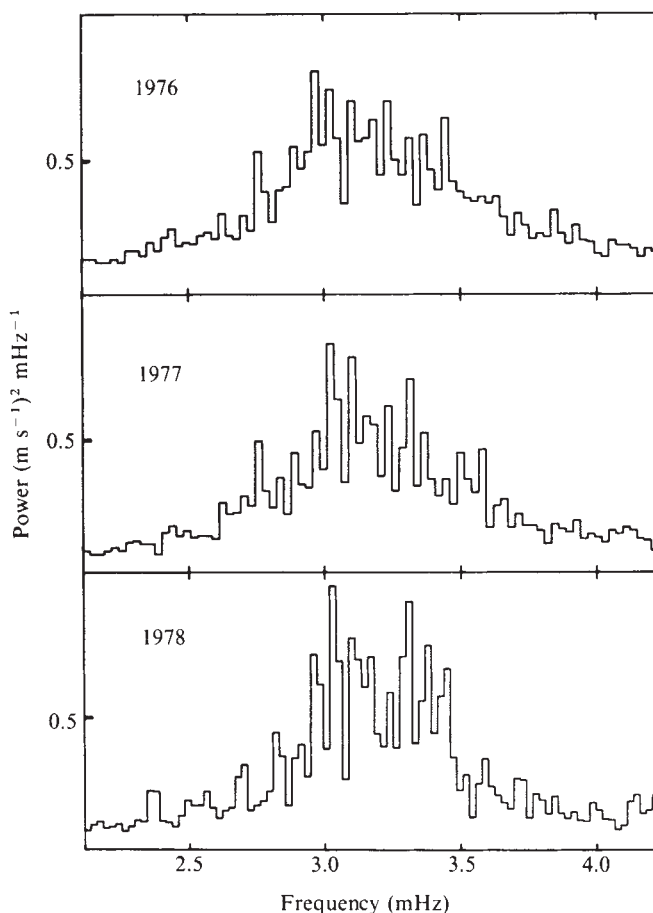


Fig. 3 Mean power spectra obtained from data collected over 33 days in 1976, 35 days in 1977 and 7 days in 1978 at Izana.

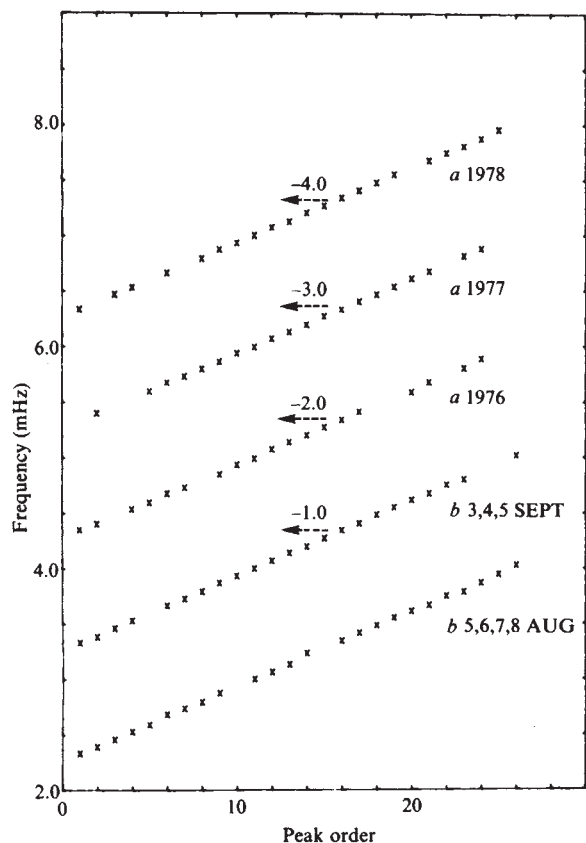


Fig. 4 *a*, Peak frequency against order plotted for the mean power spectra of 1976, 1977 and 1978. Successive plots are displaced by 1 mHz on the vertical scale as indicated. *b*, Similar plots for data determined by a superimposed epoch analysis for 4 days in August and 3 days in September 1978.

appropriately increased. The data are well fitted by straight lines and yield mean spacings of 67.6, 67.4 and 67.6 μHz for the 3 yr. Second, the mean power spectra data are screened by a high pass filter by subtracting a moving mean over three points, and then subjecting the resulting points to an autocorrelation and power spectrum analysis. In this way mean line spacings of 67.8, 68.0 and 68.0 μHz are found for the 3 yr. A cross-correlation analysis

of the 1976 and 1977 power spectra yields a correlation coefficient of 0.89 and has a maximum at zero frequency lag. This firmly establishes the consistency of the lines over the 2 yr.

As an alternative form of treatment, the data of 1978, which are of higher quality due to improvements in experimental technique, were subjected to a superimposed epoch analysis. Four consecutive days in early August and three in September were used for this study, the frequency range being restricted to that covered by the power spectrum analysis. The structure found in the power spectrum analysis was again evident and a plot of the frequency against peak order is shown in Fig. 4*b*. Straight line fits to these plots yielded mean line spacings of 67.4 ± 0.5 and 67.9 ± 0.2 μHz , respectively. The results of the superimposed epoch analysis over the four consecutive days seem to imply that the Q value of these oscillations is $>1,000$.

To investigate further the coherence of the oscillation, a power spectrum analysis of two consecutive days of data was undertaken with zeros in the data string corresponding to the times when actual data were not available. This resulted in a 32-h data string whose power spectrum is shown in Fig. 5*a*. The usual peaks in the power spectrum are clearly evident. To test for the coherence of these peaks sine waves of the indicated frequencies were subtracted from the original data by using a least squares fitting procedure to optimise the frequency, phase and amplitude of the fitted wave. The new power spectrum obtained after subtraction of these coherent fitted sine waves from the data is shown in Fig. 5*b*. This clearly demonstrates that the oscillations are coherent over the 32-h data string and thus have a $Q > 400$.

It seems likely that these lines correspond to normal modes of vibration of the whole Sun. To identify these modes with certainty, information on their spatial structure is required. An integral light spectrometer tends to average out modes of high l value with many peaks and troughs across the visible disk of the Sun⁶, thus strongly biasing the observations towards low l value modes. An approximate calculation of the sensitivity of a velocity spectrometer to various spherical harmonic modes can be found elsewhere¹³ but a more precise evaluation must include the spectral weighting function across the solar disk¹⁴. The above suggests that the observed modes are of low l value, possibly $l = 0$ and $l = 1$, with even and odd l values alternating as the value of n (number of radial nodes) increases. The observed mean spacing of 135.6 ± 0.3 μHz of next nearest lines falls in the range of spacings (130–142 μHz) predicted by solar models with uniform, as well as surface enhanced, heavy element abundances. An extrapolation of the uniform model calculations⁷

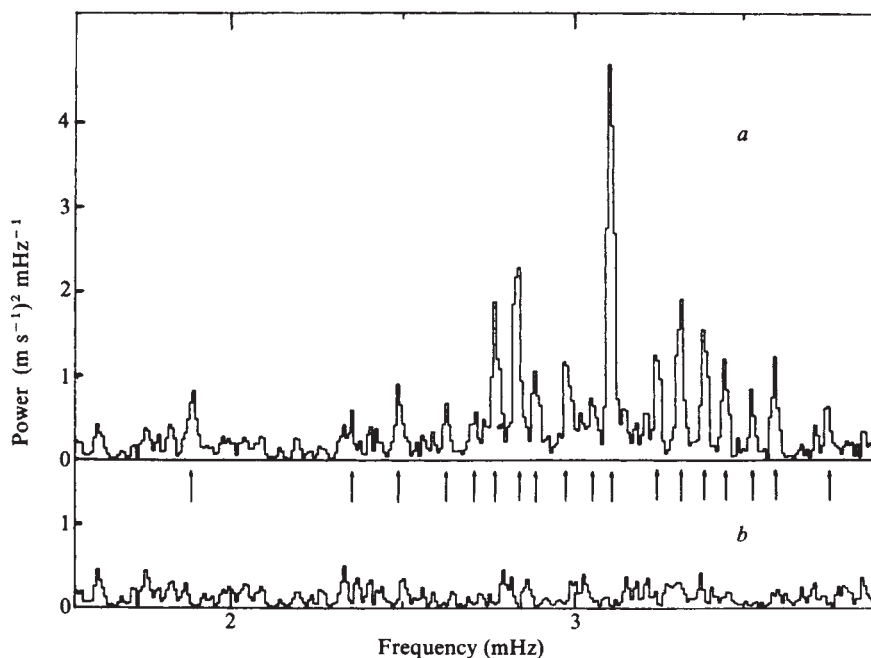


Fig. 5 *a*, Power spectra of two consecutive days of data obtained at Izana on 4 and 5 August 1978. *b*, Power spectra of the same data after subtraction of indicated discrete frequencies.

suggests that the observed spacing is consistent with a heavy element abundance of $Z \sim 0.002$. An interpolation of the non-uniform model predictions⁸, with a surface heavy element abundance of 0.02, indicates that a mean heavy element abundance of $Z \sim 0.004$ closely fits the observed spacing. Assignment of the lines to l values ≥ 2 is either not consistent with the uniform model⁷ or leads to even lower Z values for the non-uniform model⁸. This suggests that any interpretation in terms of these models implies a Z value ≤ 0.004 and a solar neutrino flux of ≤ 2.3 SNU, consistent with the observed value of 1.75 ± 0.4 SNU⁹.

Values of n can be tentatively assigned to the lines using the uniform model for which frequencies of particular modes in this region⁷ have been calculated. Using the low l value assignment and the approximate Z value limit quoted above, the observed frequencies of 2.41 and 4.04 mHz are tentatively identified as $l=0$ modes with $n=17$ and 29, respectively. Such an identification is consistent with the observed slight inequality in spacing between alternate lines. Thus, if the frequencies ν of two neighbouring $l=0$ lines are compared with that of an $l=1$ line, as expected⁷, $\frac{1}{2}(\nu_{n+1,0} + \nu_{n,0}) > \nu_{\text{odd}}$. The heavy element abun-

dance corresponding to this identification is near $Z = 0.004$.

The discrete lines are expected to be hitherto unresolved multiplets due to incomplete degeneracy of different n, l contributions. Rotation of the Sun is further expected to split the modes into $(2l+1)$ components of spacing characterised by the mean angular velocity of the interior^{6,14}. This will produce a long period amplitude modulation of the lines, some evidence for which already exists. It should be possible to resolve this fine structure by using long data strings, provided that the coherence time is adequate.

The definite assignment of the various modes is being attempted by a two-dimensional study of the solar photosphere. The amplitude variation of particular lines, as well as phase information, is being analysed further to study the excitation and damping mechanisms.

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Spurious radiation from microwave ovens

B. Anderson, R. Pritchard & B. Rowson

University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank, Macclesfield, Cheshire, UK

In agreement with the regulations of the International Telecommunication Union the manufacturers of microwave ovens are licensed to operate within the frequency band 2.45 ± 0.05 GHz reserved for industrial, scientific and medical purposes. Evidence that microwave ovens emitted significant amounts of radiation outside this licensed band and covering the reserved bands allocated to radioastronomy and communications was given in a report prepared for the Royal Society and Ministry of Technology in 1969¹. No action was taken by the licensing authorities to enforce restrictions on this spurious radiation and because the use of microwave ovens has become widespread we have made and report here a new series of measurements to assess the extent of this radiation from the ovens now in use. Our main concern has been with the spread of the oven radiation into four of the bands protected internationally for the use of radioastronomers: 1,400–1,427 MHz (21 cm H-line band); 1,660–1,670 MHz (18 cm OH band); 2,690–2,700 MHz (11 cm S-band); 4,990–5,000 MHz (6 cm C band). Although our primary interest has been to estimate the limitations which may be placed on the performance of the radio telescopes at Jodrell Bank by microwave ovens used in the vicinity, the results are relevant to the communication systems that are allocated nearby frequency bands.

Measurements have been made on three ovens from different manufacturers; two were new domestic models currently on sale and the third was a larger commercial model that had been in service for 10 years and was coated with deposits of grease and grime. As it is unwise to operate the ovens without a load, a

beaker containing approximately 300 ml of water was placed centrally on the shelf of the oven. The radiation levels were measured with a tunable receiver connected to a log-periodic antenna having nearly constant gain over the band 1–6 GHz. The receiver sensitivity was measured by means of a calibrated signal generator.

It was established that the received signal did not depend significantly on the polarisation of the antenna. The data presented here were obtained with the antenna vertically polarised and directed at the front of the oven from a distance of 3 m. Figure 1 shows an oscilloscope display of the total power received by the antenna in a bandwidth of several gigahertz (*b*), and the power received in a 7 MHz bandwidth centred on 2,695 MHz (*a*). The oscilloscope time base was triggered at a rate of 50 Hz; the exposure time was 1 s. The rotation of the

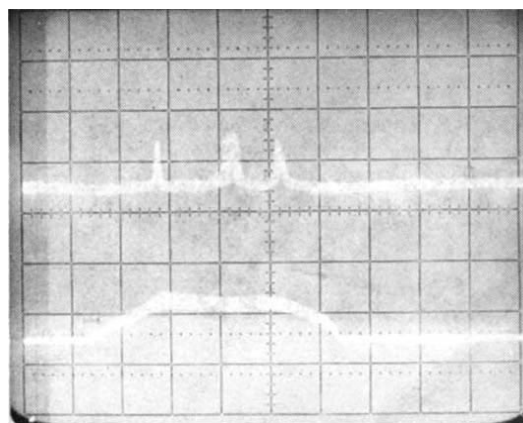


Fig. 1 The power received by the antenna as a function of time. *a*, The output of a receiver tuned to 2,695 MHz with a bandwidth of 7 MHz. *b*, The total power received by the antenna. The time base was triggered at a 50 Hz rate and the total time displayed is 20 ms.

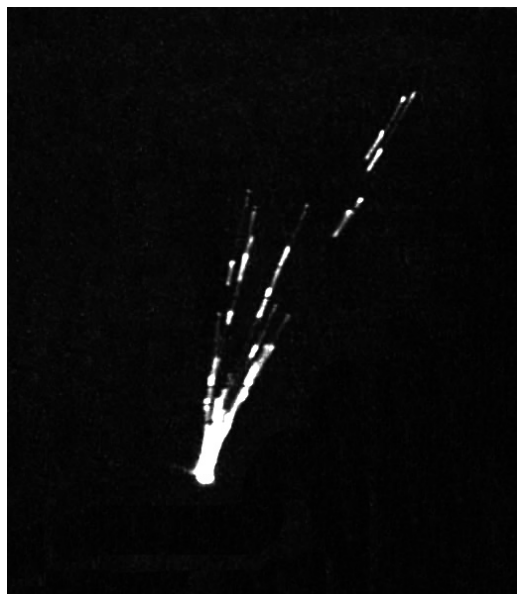


Fig. 2 A polar display of the instantaneous frequency of the major component received by the antenna. The radial distance from the bright spot is a measure of the intensity and the azimuthal angle is a measure of the frequency. One rotation covers 88 MHz.

metal paddle inside the oven was inhibited during the exposures. Movement of the paddle by hand affected the amplitude of the lower trace and caused the position of the short pulses on the top trace to move back and forth in time. Tuning the receiver centre frequency over tens of MHz had the same effect on the short pulses.

Essentially the same behaviour was observed wherever the receiver was tuned between 1 and 6 GHz except that the top trace became similar to the bottom trace near and below the nominal oven operating frequency of 2.45 GHz and also near the second harmonic at 4.9 GHz. With the paddle stationary, it was possible to find restricted regions of the spectrum where the radiation was below our limit of detection in a 7 MHz bandwidth but these quiet regions were much less frequent if the paddle was rotating.

Figure 2 shows a polar display of the total power (radial distance) versus instantaneous frequency (position angle of the trace with respect to the bright central spot) received by the antenna. A 90° difference in position angle corresponds to a frequency difference of 22 MHz. Each 'finger' on the photograph is a mode of the oven and the trace intensity is propor-

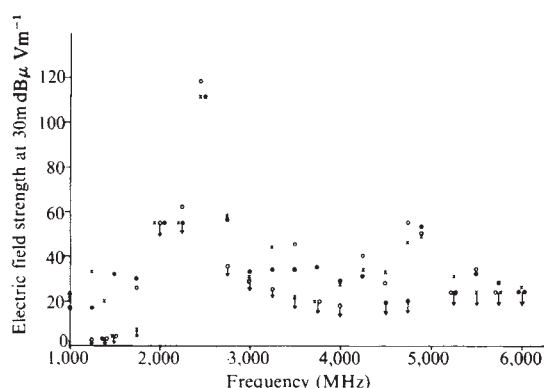


Fig. 3 Electric field strength at a distance of 30 m as a function of frequency for the radiation from three microwave ovens: ○, used commercial oven; × and ●, new domestic ovens.

tional to the probability of finding the output at that power and frequency. The largest observed separation of the modes was ~30 MHz. It seems, therefore, that the major components of emission fall within the frequency limits specified.

Figure 3 shows the peak value of the electric field components of the spurious emissions from the ovens measured in a 30 kHz bandwidth and normalised to a range of 30 m (assuming an inverse square relationship for power). The measurements are plotted for all three ovens at 0.25 GHz intervals between 1 and 6 GHz. These data are in good agreement with earlier Post Office measurements of a completely different set of ovens manufactured between 1962 and 1968 (ref. 1).

The interference effects from microwave ovens on radio telescopes working in the frequency bands allocated for astronomical purposes and listed above have been calculated as follows. The receiving system on a telescope generates a noise signal, P_N , in the receiver bandwidth, B , equivalent to a mean input power given by

$$P_N = kT_s BW$$

where k is Boltzmann's constant and T_s is the effective system noise temperature in degrees Kelvin. If the receiver output is averaged for a time τ , the input power can be established with a statistical uncertainty

$$\delta P_N = kT_s (B/2\tau)^{1/2}$$

Signals from radio sources that add more power to the receiver than δP_N can be detected with a statistical confidence dependent on the factor by which the added power exceed δP_N in the usual way for a normal error distribution.

Table 1 Specification of the maximum levels of radiation in the 1 to 6 GHz band from interfering sources assuming T_s is 30 K

A	Mean power from interfering source	$< 2 \times 10^{-20}$ W in 10 MHz bandwidth	} assuming $\tau = 2 \times 10^3$ s
		$< 0.65 \times 10^{-21}$ W in 10 kHz bandwidth	
		$< 0.65 \times 10^{-22}$ W in 100 Hz bandwidth	
B	Peak power averaged over 1 ms	$< 2.8 \times 10^{-17}$ W in 10 MHz bandwidth	$(\delta t = 10^{-3}$ s)
C	Peak power averaged over 1 ms of a repetitive signal with period > several milliseconds	$< 2.8 \times 10^{-19}$ W in 10 MHz bandwidth	$(N\delta t = 10$ s)

Many astronomical radio sources have continuous spectra so that the telescope receives from the source a power proportional to the receiver bandwidth. A larger value of B then increases the signal-to-noise ratio despite the proportionality of δP_N to $(B)^{1/2}$. A long integration time also improves the signal-to-noise ratio for those sources for which the intensity is not a function of time. In the case of pulsars where the intensity of the source is a function of time, τ is limited to the desired time resolution, δt , if the purpose is to study the structure of a single pulse, or to $N\delta t$ if the purpose is to study the integrated profile of N pulses with time resolution δt .

The power received from interfering sources is not generally constant as a function of time: either the source emission varies or the telescope sweeps its sidelobe structure over the source as it tracks an astronomical object. The net result in either case is to increase the fluctuations in the receiver output above the natural limit and hence impair the sensitivity of the radio telescope. The power received from interfering sources must be below δP_N if the performance of the telescope is not to be affected.

On this basis, limits may be placed on the tolerable power levels of interference for a radio telescope. These limits are given in Table 1 assuming that the system noise temperature, T_s , is 30 K. It must be stressed that Table 1 is based on assumptions and limits which are typical of contemporary systems used in radioastronomy and are, in fact, 10 times less severe than the

CCIR Report on the protection criteria for radioastronomy². On the other hand, we assume that there is only one source of interference which is not likely to be the case if microwave ovens become widespread.

The power (P_i) received from an interfering source by a radio telescope depends on the equivalent isotropically radiated power (EIRP) of the source in the direction of the telescope, the range of the source from the telescope (R_i), and the gain of the telescope (G_i) in the direction of the source. The relationship between these parameters is

$$P_i = (EIRP)G_i(\lambda_i/4\pi R_i)^2$$

where λ_i is the wavelength of the radiation. The appropriate G_i to use in the case of a ground-based source of interference is that of a far-out sidelobe of the telescope response for which $G_i \sim 1$ (a -63 dB sidelobe for a 80-m telescope at $\lambda \approx 12$ cm).

Measurements were made on the interference from one of the domestic ovens in the protected frequency bands for radioastronomy shown in Table 1. The results are shown in Table 2 together with a comparison with limits A and C of Table 1. These results have been converted to levels received by a radio telescope at a range of 1 km assuming that G_i is 1 and that power flux density is $\propto R_i^{-2}$. Measurements were also made 50 MHz on either side of each of these bands as it was thought that there might be nulls in the spectrum that were oven-load dependent or not repeatable from oven to oven.

The data presented in Table 2 show that spurious emissions from microwave ovens present a serious danger to radioastronomical researches and at some parts of the spectrum they are at a level that would degrade the performance of a point-to-point radio link where the possibility of having an oven in the main beam of, and close to, a receiver cannot be discounted.

The power levels given in Table 2 for the four protected frequency bands have been used to derive the data given in Table 3 for the influence of a single oven operating at various distances from a radio telescope with a sidelobe level with a gain equivalent to an isotropic radiator. Table 3 makes no allowances for any screening of the oven that may be provided by the walls of buildings, or other obstructions. The Post Office report¹ estimates that up to 30 dB attenuation of the signals may be caused by such obstructions but this form of screening cannot be relied on—a glass window is relatively transparent to microwaves and an average kitchen window would allow spurious emissions from an oven to escape unattenuated over a wide range of angles.

The emission of significant microwave power over such a wide spectral range seems to arise from the method of generation of the power coupled with the ineffective shielding at the seals of the oven door. The source of the microwaves is a magnetron producing 1–2 kW of power and operating from rectified but

Table 3 Range of detectability as a function of frequency of the spurious emission from a microwave oven (line of sight without local screening)

Frequency (MHz)	Range in a sidelobe with $G_i = 1$ (km)	Frequency (MHz)	Range in a sidelobe with $G_i = 1$ (km)
1,365	1.6	2,645	7
1,415	3.5	2,695	25
1,465	2.7	2,745	27
1,620	1.1	4,945	8
1,665	<0.6	4,995	~0.8
1,715	<0.6	5,045	~0.5

unsmoothed a.c. mains. The power is generated for approximately half of each supply cycle. The magnetron drives a load that is deliberately disturbed by a rotating metal paddle in the oven with the consequence that both the instantaneous frequency at which it oscillates and the power that it delivers are functions of time. The instantaneous frequency lies in the assigned band of $2,450 \pm 50$ MHz but modulation sidebands exist and it is these which give the out-of-band emissions. It is also possible that some of the spurious emissions are caused by instabilities in the electron distribution inside the magnetron leading to the generation of unwanted frequencies^{3,4}.

The primary source of leakage of radiation is from the seals around the door which fail to confine the microwave radiation to the inside of the oven⁵. These seals are non-contacting and seem to consist of a resonant, quarter-wavelength choke nominally tuned to 2,450 MHz followed by microwave absorbing material. The seals are sufficiently effective in the assigned band of 2,450 MHz to satisfy the safety regulations appropriate to the UK⁶, but they fail to give adequate out-of-band suppression to prevent possible interference with other services authorised to operate within the 1–6 GHz frequency band.

The measurements of the spurious emission from microwave ovens over the frequency band 1–6 GHz are in substantial agreement with those reported previously¹. In particular these emissions cover at least four of the frequency bands which are allocated by the ITU to radioastronomy. Increasing use of the ovens in the vicinity of radio astronomical installations and of communication systems working in this frequency range would place serious limitations on the operation of these installations. Whereas the Radio Regulatory Department of the Home Office place stringent limits on the amount of the spurious radiation which is allowed from communication systems no such limits seem to be imposed on the amount of the out-of-band radiation allowed from microwave ovens.

The design of the ovens on sale in UK is governed by a directive⁶ which recommends an upper limit for continuous exposure of 10 mW cm^{-2} and specifies that the test equipment should be capable of measuring to 1 mW cm^{-2} and preferably to 0.5 mW cm^{-2} . Furthermore it is specified that the equipment used for the measurements should be "relatively insensitive outside its declared frequency band". The measurements reported here reveal that this specification (laid down in 1960) is not sufficient. The regulations need revision so that the use of microwave ovens is not allowed if the amount of the emitted radiation outside the licensed band of $2,450 \pm 50$ MHz can cause harmful interference to authorised radio services.

Table 2 Levels of interference from a microwave oven at a distance of 1 km for radiation received in a far-out sidelobe ($G = 1$)

Frequency (MHz)	Mean power into receiver at 1 km, 10 MHz BW (W)	Peak power into Rx at 1 km 10 MHz BW (W)	Factor by which mean exceeds limit A of Table 1	Factor by which peak exceeds limit C of Table 1
1,365	5×10^{-20}	2.5×10^{-18}	2.5	9
1,415	2.5×10^{-19}	5×10^{-18}	12.5	18
1,465	1.5×10^{-19}	7.5×10^{-18}	7.5	27
1,620	2.5×10^{-20}	2.5×10^{-18}	1.25	9
1,665	$<0.75 \times 10^{-20}$	$<2.5 \times 10^{-20}$	<0.4	<0.09
1,715	$<0.75 \times 10^{-20}$	$<2.5 \times 10^{-20}$	<0.4	<0.09
2,645	1×10^{-18}	1.5×10^{-16}	50	530
2,695	1.25×10^{-17}	2.5×10^{-15}	625	8,900
2,745	1.5×10^{-17}	2×10^{-15}	750	7,100
4,945	1.25×10^{-18}	2.5×10^{-17}	62.5	89
4,995	$\sim 1.5 \times 10^{-20}$	$\sim 1.5 \times 10^{-18}$	~0.75	5.4
5,045	$\sim 0.5 \times 10^{-20}$	$\sim 0.5 \times 10^{-18}$	~0.25	1.8

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Corrosion micromorphology of noble metal alloys and depletion gilding

A. J. Forty

Department of Physics, University of Warwick, Coventry, UK

Noble metal alloys commonly undergo selective dissolution during anodic corrosion whereby the less noble component is dissolved preferentially. This is particularly interesting, as it leads to a depletion of the less noble species not only at the surface of the metal but also at an appreciable depth within it, implying that a mass transport of atoms is occurring either through the bulk of the crystal lattice or across the surface to support the continuing dissolution. Experiments in which the micromorphology of silver-gold alloys is observed during such corrosive treatment have shown that surface diffusion of gold plays an important part in this process. As surface silver atoms are dissolved the residual gold atoms reform into gold-rich islands so that fresh silver atoms are continuously exposed to the environment layer by layer. These observations will be described in detail elsewhere¹. Here the mechanism of surface re-arrangement is considered, which leads to the accumulation of gold-rich surface regions, and throws a new light on the ancient craft of depletion gilding.

Depletion gilding, or gold colouration (*mise-en-couleur*) is the process by which the surface of a dilute gold alloy becomes gold-enriched by the selective removal of the less noble component. This usually involves annealing the alloy or immersing it in a corrosive bath, or some combination of these treatments. It is well established that the Indians of pre-Columbian Central America had invented such a technique for the colouration of castings prepared from copper-gold alloy², commonly referred to as tumbago. Their colouring methods involved either the formation of copper oxide by heating in air, followed by chemical dissolution of the oxide or the slow removal of copper from the surface of the alloy in a corrosive bath. The 'spongy' gold layer so-formed was then consolidated by burnishing. It is thought that similar processes were used by North Peruvian craftsmen to colour objects fabricated from silver-based alloys³. However, the details of these processes, particularly the nature of the chemical reagents used, are still matters for conjecture.

A very closely related process for the colouration of silver-gold alloys, known as cementation, is known to have been used

by European and Near Eastern goldsmiths since before the early Middle Ages. The object is packed in a reactive powder (invariably containing salt) which, when heated, forms soluble chlorides with silver and other impurities in the alloy, leaving the surface of the object gold-rich.

From the metallurgical point of view the most interesting aspect of these ancient crafts is the process by which the gold becomes parted from its alloys by the selective corrosion of the less noble components. Nitric acid has been used for parting silver and gold in Europe since the twelfth or thirteenth century. However, apart from the very superficial observation that nitric acid preferentially dissolves the silver, leaving the surface of the alloy coated with a spongy gold layer⁴, there has been no detailed study of the metal physics of these surface enrichment processes. The micromorphological observations reported here may, however, provide some new insight into the understanding of these ancient technologies.

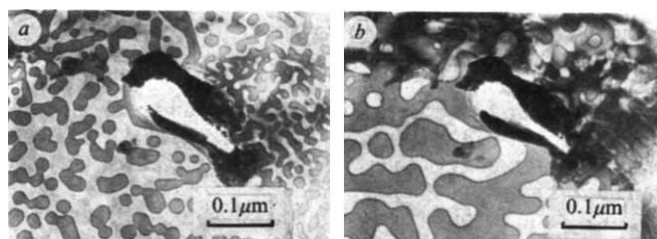


Fig. 2 Transmission electron micrograph of 50:50 Ag-Au alloy: *a*, after corrosion for 30 s in 50% nitric acid; *b* after corrosion for 60 s in 50% nitric acid.

Figure 1 shows a transmission electron micrograph of a single crystal thin film of a 50:50 silver-gold alloy after a small amount of corrosion in a 50% aqueous solution of nitric acid. The film, originally structureless, apart from a small number of dislocations, twins and double-positioning boundaries⁵, has become transformed into a maze-like structure of island of more strongly absorbing material separated by more transparent channels. The selected area X-ray analysis facility available in the electron microscope, has shown that the island regions of the film are gold-rich compared with the channels, which is consistent with the suggestion that the islands are formed during corrosion by the aggregation of the gold residue on the surface after selective dissolution of silver. Figure 2 demonstrates how the islands grow and the channels between them shrink as the corrosion proceeds, and Fig. 3 shows how annealing at only 720 K after an initial exposure of the alloy to nitric acid causes the islands to grow and merge together to form a connected gold-rich surface layer with residual pits where the merger is incomplete. Similar observations on other silver-gold alloys and also copper-gold alloys over a wide range of compositions and exposed to a wide variety of acid environments have confirmed that the island nucleation and growth is common.

These corrosion-induced micromorphological changes can be explained on the basis of a model in which the selective dissolution of silver produces an atomic roughening of the surface

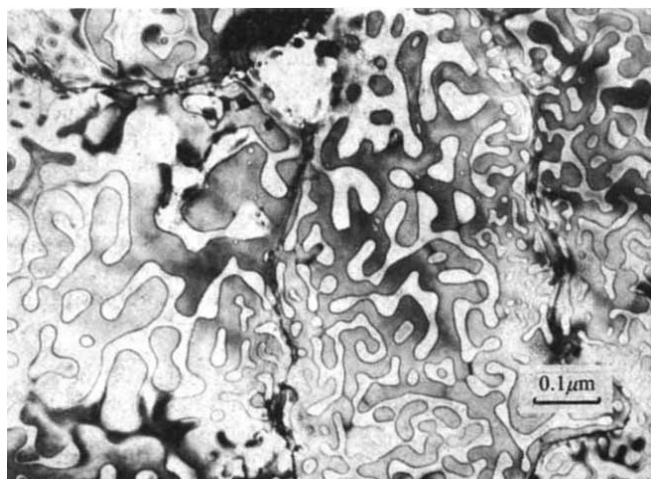


Fig. 1 Transmission electron micrograph of 50:50 Ag-Au alloy after corrosion in 50% nitric acid, showing island and channel morphology.

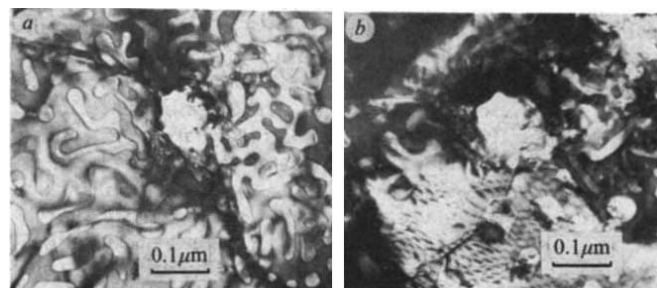


Fig. 3 Transmission electron micrograph of 50:50 Ag-Au alloy: *a*, after corrosion for 60 s in 50% nitric acid and then *b*, after annealing for 60 s at 720 K.

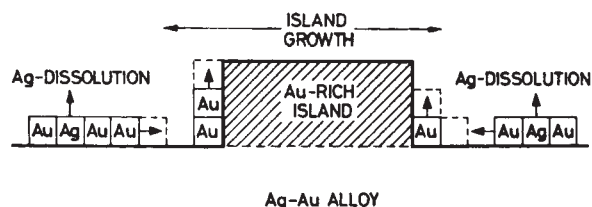


Fig. 4 Gold island growth by the surface disordering, reordering mechanism.

of the alloy (surface disordering). The surface disorder may be regarded as a random distribution of surface silver vacancies or surface gold adatoms, depending on whether the alloy is gold-rich or silver-rich. As the corrosion proceeds the surface reorders by island nucleation and growth from the high concentration of gold adatoms. This process is illustrated in Fig. 4. Finally, the islands grow into an interconnected structure resulting in a very thin epitaxed surface coating of gold.

Although these observations are made on single crystal, high purity alloys, specially prepared for corrosion studies⁵, the surface disordering, reordering model can readily be extended to the more practical situation of depletion gilding of castings. The micromorphology of the gold-rich surface layer will vary in detail according to alloy composition, type and strength of acid used, local changes of composition and alloy microstructure at grain boundaries¹, but the final result is expected to be a thin tenacious coating of gold with imperfections which can be subsequently removed by a burnishing treatment.

It would be interesting to carry out a detailed micro-morphological study of this kind on binary and ternary silver, copper and gold alloys after corrosion in reactive environments similar to those thought to be used by the ancient Indian goldsmiths.

I thank Professor Colin Goodman for drawing my attention to the Royal Academy Exhibition *The Gold of El Dorado* which caused me to connect my work on the corrosion morphology of silver-gold alloys with the art of depletion gilding.

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Incorporation of elemental sulphur in coal as organic sulphur

Daniel J. Casagrande* & Lily Ng

Governors State University, Park Forest South, Illinois 60466

Organic sulphur can represent over 50% of the total sulphur content in coal¹, but little is known about the nature of this organic sulphur because of the chemical complexity of the coal structure and heterogeneity of the maceral assemblages forming the coal matrix. This report focuses on the mode of incorporation of organic sulphur in the very early stages of the coal-forming process. Some organic sulphur in coal originates from the sulphur-containing amino acids that are present in source plants in the peat-forming stage, but we demonstrate here that some of the organic sulphur in coal can actually originate from an inorganic sulphur source, such as elemental sulphur.

The coal-forming process is a geochemical continuum that includes: peat → lignite → bituminous coal → anthracite coal. Contemporary coal progenitors can be found in peat-forming systems of the Okefenokee Swamp, Georgia and the Everglades Swamp, Florida². Each peat-forming system is a dynamic community composed of plants, surface litter, water, minerals, microorganisms and peat; in the case of the Okefenokee, its present vegetation is similar to that which gave rise to the huge Braunköhle accumulations in Germany and some of the lignites in the Dakotas^{3,4}.

Virtually all the sulphur in some coals can be accounted for in the peat-forming stage of coal formation⁵. The forms of sulphur that have been found in peat include pyrite, sulphate, hydrogen sulphide, elemental sulphur, carbon bonded (C-S) sulphur, and ester sulphate (C-O-S)^{5,6}. Organic sulphur is the major variety of sulphur in virtually all peats studied: it represents over 60% of the total sulphur. One origin for the organic sulphur in peat is the sulphur-containing amino acids such as methionine, cystine and cysteine that are contributed to the sediment by decaying plants and associated microflora and microfauna, but less than 40% of the total organic sulphur can be accounted for as sulphur associated with amino acids⁷. As a function of depth, fewer sulphur-containing amino acids were observed in peat from a marine environment, even though total organic sulphur increased substantially with depth.

Initially, it was thought that the sulphur-containing amino acids were being incorporated via chemical reaction into the various organic fractions of the peat, such as humic and fulvic acids. Yet, in many peat profiles, organic sulphur increased to levels that were substantially greater than the source plant materials and certainly considerably higher than that found in the upper levels of the peat profile, suggesting other sources of organic sulphur in the peat. Substantial amounts of elemental sulphur are observed in some peat-forming systems⁵, but virtually none in coal¹. The elemental sulphur is probably of micro-bial origin⁸.

To test the idea that elemental sulphur serves as a source of organic sulphur in peat, and hence coal, low sulphur peat (0.10%) from the Okefenokee Swamp was fractionated to produce humic acids. Humic acids were chosen for the reaction since they are recognised progenitors of some of the more important macerals in coal and the organic sulphur of coal is associated with the maceral assemblage. A 1-g aliquot of the prepared humic acid was placed in a round bottom flask and 100 ml of chloroform and 5 μ Ci of ³⁵S were added to each flask. The contents of the flasks were refluxed for 6 to 48 h, then cooled, filtered and rinsed thoroughly with chloroform and then 1M HCl (chloroform removed any unreacted elemental sulphur, and the HCl dissolved any FeS that may have formed). The total ³⁵sulphur and inorganic ³⁵sulphur were determined using the Johnson-Nishita procedure⁹ as modified by us^{5,6}, and organic ³⁵sulphur by difference. For total sulphur analysis, all the sample sulphur was oxidised to sulphate, and then the oxidised sample was placed on a Johnson-Nishita apparatus; the sulphur in the sample was subsequently reduced to H₂S via a HI-reducing solution; the H₂S was then carried via nitrogen into a zinc/sodium acetate solution, where the ³⁵sulphur became bound as Zn³⁵S. An aliquot of this solution was mixed with Insta-Gel and counted in a Packard liquid scintillation counter. For the analysis of inorganic sulphur, the oxidation step, in the sample preparation, was omitted; organic sulphur was determined by difference. It should be noted that any adsorbed sulphur would be detected in the analysis used for inorganic sulphur.

When ³⁵S was allowed to react with the prepared humic acid fraction there was a near linear increase in the organic sulphur content of the humic acid, as a function of time (Fig.1). The conditions of the experiment were mild enough and the chemical reactants were appropriate enough to warrant serious consideration of this reaction as a potentially important source for some of the organic sulphur found in coal. The exact chemical mode of incorporation of elemental sulphur into humic acid is

* Present address and address for reprints: Exxon Production Research Company, Box 2189, Houston, Texas 77001.

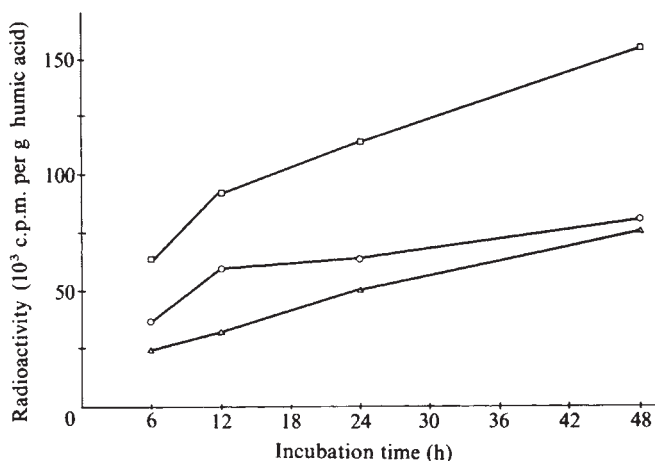


Fig. 1 Uptake of elemental sulphur in the humic acid fraction of Okefenokee peat. □, Total sulphur; ○, inorganic sulphur; △, organic sulphur.

not clear. Sulphur associated with humic acids in marine environments has been shown to be isotopically light, suggesting a microbial origin^{8,10}. Elemental sulphur in sediments is thought to originate from the microbial oxidation of H_2S , and H_2S is known to originate from the microbial use of sulphate as a terminal hydrogen acceptor^{8,11}.

Further evidence of interactions of elemental sulphur with the organic matter in peat comes from the findings that elemental sulphur will react with certain amino acids¹², cholesterol¹³ and ethyl benzene¹⁴ to produce organic sulphur compounds. However, in all these cases, reactions were carried out at temperatures at 130°C or above; in some cases, closed tube conditions with the addition of oxygen and catalysts were used. At these temperatures it is possible that H_2S was formed and hence was the actual reactant chemical as opposed to the original elemental sulphur. Experiments performed in the present study more closely approximate the environmental conditions that elemental sulphur and peat are subjected to in their diagenetic and metamorphic routes to coalification. The exact mechanism for the reaction of elemental sulphur with peat is not known—many carbonium ion and free radical mechanisms are possible, even at the relatively low temperatures of this experiment¹⁵.

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H_2S incorporation in coal precursors: origins of organic sulphur in coal

D. J. Casagrande*, G. Idowu, A. Friedman, P. Rickert, K. Siefert & D. Schlenz

Governors State University, College of Arts and Sciences, Park Forest South, Illinois 60466

Of the forms of sulphur in coal—organic, pyrite, and sulphate—the organic sulphur component is the most difficult to remove before coal can be used. Organic sulphur, which is generally regarded as sulphur in a carbon-sulphur linkage, can represent >50% of the total sulphur found in some coals¹. We describe here an investigation into the origin of the organic sulphur in the coal-forming sequence which includes: peat → lignite → bituminous coal → anthracite coal. Because of the chemical heterogeneity and associated chemical complexity of working with either bituminous or anthracite coals, the earliest stage of coal formation, that is the peat-forming stage, was studied. Modern progenitors of coal from the Okefenokee Swamp were studied, as these peat-forming systems closely approximate ancient systems that eventually gave rise to huge coal deposits²⁻⁴. While amino acid sulphur from source plants is an important progenitor of organic sulphur in peat, it was found that H_2S can react with the organic matter in peat to produce organic sulphur—a source of organic sulphur in coal that has not been previously discussed.

The peat-forming stage is a crucial time for sulphur incorporation in the coal-forming process; virtually all of the organic and pyritic sulphur in high and low sulphur coals can be accounted for at this stage^{5,6}. Modern peat-forming systems, such as the marine-derived mangrove peats in the Florida Everglades, can contain as much as 10% total sulphur, of which 30-50% is organic sulphur. Sulphur must accumulate in peat as its content is sometimes an order of magnitude higher than that found in associated plants. Sulphur-containing amino acids, such as cysteine, methionine and cystine, found in both higher plants (source materials for the peat) and microorganisms (degraders of the peat), have been viewed as the source of organic sulphur found in coal⁶. Analysis of sulphur-containing amino acids in mangrove-derived peats shows that only 20-40% of the organic sulphur in peat is present as sulphur incorporated in amino acids^{7,8}. Thus, the source of a major portion of organic sulphur in coal formation is in the peat-forming stage, yet the origin of this organic sulphur has not been fully explained. In walks through the peat during a field trip, a strong smell of H_2S was common; the source of this H_2S is probably microbial. *Desulfovibrio* occurs in anoxic environments and this bacterium uses sulphate as a terminal hydrogen acceptor, producing H_2S during the process⁹. In a series of experiments in our laboratory it was observed that as much as 4 mg S per kg wet peat per day (as H_2S) was produced from mangrove-derived peats.

These observations, and the fact that the organic sulphur increase in peat is not necessarily related to the associated source plants, caused us to design a series of experiments to evaluate the possibility that some of the organic sulphur found in coal may derive from the interaction of H_2S with the organic matter in peat. Aliquots from a large sample of an homogenised wet peat sample were placed in a series of desiccators; the atmosphere over the peats was evacuated using a water pump. The peat used in this study originated from core material in the Chesser Prairie area of the Okefenokee Swamp; it had a sulphur content of 0.25% on a dry basis. An $H_2^{35}S$ generating system was constructed that allowed an $H_2^{35}S/N_2$ atmosphere to be introduced over each peat sample. In operation the peat was blanketed in the desiccators with a $H_2^{35}S/N_2$ atmosphere as follows:

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Table 1 The distribution of labelled organic sulphur in organic peat fractions

Time (h)	Water soluble	Benzene/methanol	Humin	Humic acid	Fulvic acid
72	5.64	—	81.84	12.03	0.49
168	3.90	0.007	40.84	55.10	0.16
504	1.08	0.14	7.59	90.65	0.44
840	1.00	0.11	25.19	73.63	0.04
Mean	2.90	0.09	38.86	57.85	0.28

(1) Na_2^{35}S was put into a two neck round bottom flask, which had one side adapted for the introduction of H_3PO_4 and the other side adapted for a gas inlet/outlet; (2) the system was purged with N_2 gas; (3) H_3PO_4 was introduced on top of the Na_2^{35}S ; (4) the entire system was closed to allow for H_2^{35}S build-up; (5) stopcocks were opened that would allow for the passage of nitrogen though the H_2^{35}S generating chamber and conduction of the $\text{H}_2^{35}\text{S}/\text{N}_2$ mixture into the previously evacuated desiccators; and (6) the desiccator atmosphere was filled with N_2 until ambient pressure was reached.

After various intervals (72–840 h) an individual desiccator was opened and the peat was fractionated into five components; water soluble, benzene/methanol soluble, fulvic acid, humic acid and humin. Each fraction was then analysed for both total and inorganic labelled sulphur. Note that the procedure used to analyse inorganic sulphur would also measure any hydrogen ^{35}S sulphide that was adsorbed along with any elemental ^{35}S sulphur, ^{35}S sulphate, ^{35}S sulphides, ester ^{35}S sulphate, and so on, but does not measure carbon bonded (organic) ^{35}S sulphur. Thus organic ^{35}S sulphur¹⁰ was calculated as the difference between total ^{35}S sulphur and inorganic ^{35}S sulphur.

Table 1 shows the percentage of total organic ^{35}S sulphur activity in the peat as a function of organic fraction and as a function of time. There are several interesting points: (1) H_2S reacts with peat to produce organic sulphur; (2) H_2S is incorporated into the organic sulphur fraction at room temperature and it occurs in a relatively short period of time—compatible with diagenetic processes occurring in modern organic sediments; (3) the humic acid fraction—an important precursor to coal maceral(s)—shows the largest amount of organic sulphur incorporation, even though it represents <20% of the total organic matter in peat; (4) the humin fraction, which represents 70% of the total organic matter, has an average of only 38.8% of the total organic sulphur associated with it; (5) there is little organic sulphur incorporation into the water soluble fraction—the water soluble fraction represents a pool of 'free constituents' which should be available for reaction; (6) little organic sulphur incorporation is found in the fulvic acid fraction—previous studies have shown that 75% of the sulphur associated with the fulvic acid fraction is in the form of ester-sulphate⁸. A plot of the uptake of H_2^{35}S as organic sulphur in the humic acid fraction, showed monotonic production of organic sulphur in the organic matter as a function of time.

The chemical nature of the bonding involved in the reaction of H_2S with organic matter is difficult to assess. It is possible that H_2S is converted to another species, which is the reactive agent^{11–13}. However, there are reports which demonstrate H_2S incorporation in (1) petroleum¹⁴, (2) organics in natural waters¹⁵, (3) non-sulphur-containing amino acids¹⁶ and (4) unsaturated fatty acids¹⁷. Also, Kaplan and co-workers have indicated that the sulphur associated with marine-derived humic acids is isotopically light sulphur which is microbially-derived¹⁸. The present study certainly supports this view and points to at least one of the microbially-derived sources for some of the organic sulphur in coal. The study also shows that important pre-maceral materials, such as humic acids, obtain an organic sulphur component at a very early point in their diagenetic history from a source that is not originally carbon-bonded. Finally, the importance of this type of reaction in the overall biogeochemical cycle of sulphur needs further investigation.

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Are solar spectral variations a drive for climatic change?

James B. Pollack, William J. Borucki & Owen B. Toon

Space Science Division, NASA Ames Research Center, Moffett Field, California 94035

Observations of the Sun from satellite and rocket platforms have provided good evidence that solar radiation varies noticeably in the UV portion of the spectrum over a solar rotation period of 27 d^{1,2}. There is also less conclusive evidence for such variations on the time scale of the 11-yr sunspot cycle^{1,2}. We investigate here the possible effects of these UV variations on atmospheric ozone content and climate for time scales encompassing the 27-d solar rotation period, the sunspot period, twice this period (solar magnetic period) and much longer times. We conclude that (1) solar UV variations on time scales of weeks to months can occasionally perturb total ozone and stratospheric temperatures by noticeable amounts, but they result in only very minor changes in the troposphere; (2) the above cited estimates of UV variation over the 11-yr solar cycle are inconsistent with measured changes in total ozone; (3) with UV variations being limited by long-term trends in total ozone, only very small variations in surface temperature are expected over the 11-yr solar cycle, but possibly significant ones could occur over the 22-yr cycle; (4) solar spectral changes might be partially responsible for century-long variations in climate, which have been empirically correlated with changes in solar activity^{3,4}.

To distinguish clearly the solar spectral change mechanism for climate modification from ones involving luminosity changes, we alter the visible spectrum slightly ($\approx$ few tenths %) so that the integrated solar radiant output is an invariant. We note that very severe upper bounds (0.03%) have been placed on the variation of the solar constant on time scales of months⁵, despite the occurrence of measurable UV variations on this same time scale^{1,2}. Somewhat larger upper limits have been set on the change in solar luminosity between 1969 and 1976 (0.15%) (ref. 6) and from 1923 to 1952 (0.17%) (ref. 7).

Our studies of the relationship between solar UV variations and atmospheric ozone content and atmospheric temperatures differ from others⁸⁻¹⁰ in that we estimate the impact of such variations on tropospheric temperatures, we hold the total luminosity constant, and we examine the dependence of the ozone variations on the forcing period.

The calculations were performed with a three-step iterative procedure for UV variations having a period long compared with the time required for the ozone profile to reach a steady-state solution (~ 1 yr). First, a one-dimensional time-marching photochemical model with vertical transport and a fixed temperature profile was used to determine the temporal variation of the ozone and nitrogen dioxide concentrations from the ground to 120 km altitude¹¹. Next, the results of the photochemical calculations were used in a numerically accurate, vertically inhomogeneous, multiple scattering computer program to determine the globally averaged, solar energy deposition profile¹². Finally, this profile was incorporated into a one-dimensional radiative-convective equilibrium program to evaluate the globally averaged temperature structure¹³. Because of the temperature dependence of the photochemical rate coefficients, we repeated the three-step calculations several times, with the temperature estimates obtained from the third step used as starting conditions.

A different procedure was followed for UV variations having a period less than the ozone relaxation time scale. In such cases the driving period is much smaller than the thermal inertia time constant for the land-ocean-troposphere system (~ 5 yr (ref. 14)). Thus, little change in tropospheric or surface temperatures will occur. For these cases, we used the same one-dimensional time-marching photochemical model, but with a parameterised radiative cooling and heating capability that provided estimates of temperature perturbations in the stratosphere and mesosphere¹⁵.

As we neglect a large number of feedback processes that could alter the final answer¹⁶, our results should be viewed simply as an exploratory study of the sensitivity of the Earth's climate to solar spectral variations. For example, we do not attempt to vary the properties of tropospheric water clouds in response to the UV ozone and temperature changes nor do we allow for dynamical effects.

There are two mechanisms that might cause solar UV variations, each being possibly pertinent for different time periods. One manifestation of solar activity is a region called a plage that covers a small fraction of the surface and is hotter than the rest of the Sun at the same altitude¹⁷. Because the spectral irradiance in the UV is on the Wien tail of the black-body function, much larger variations can occur in the UV than in the visible due to changes in the area covered by plages. We model this situation—essentially the sum of two black-body functions—by setting the fractional change in the UV spectral irradiance equal to $C \exp(-b(1/\lambda_0 - 1/\lambda)) \sin(2\pi t/P)$, in accord with available broad band observations of solar UV variations over a solar rotation period^{1,2} where C and b are constants; λ and λ_0 are wavelength and a reference wavelength (0.175 μm), respectively; and t and P are the time and period, respectively.

The increase of temperature with altitude above the solar photosphere is thought to be due to the upward propagation of energy (such as acoustical waves) from the solar convection zone that is located right below the photosphere¹⁸. Conceivably, on long time scales ($> \text{yr}$), solar activity variations may be accompanied within the convection zone by variations in the division of the net solar flux between radiative and turbulent net fluxes. If so, alterations in the vertical solar temperature structure, particularly near the solar temperature minimum and at higher altitudes, may occur, without there necessarily being a change in the solar luminosity. Both because UV irradiance is produced near the temperature minimum and because it is situated on the Wien tail of the black-body function, such alterations of temperature structure would preferentially affect the UV output, with the magnitude of the spectral variation depending on the altitude from which the irradiance originates. We model this

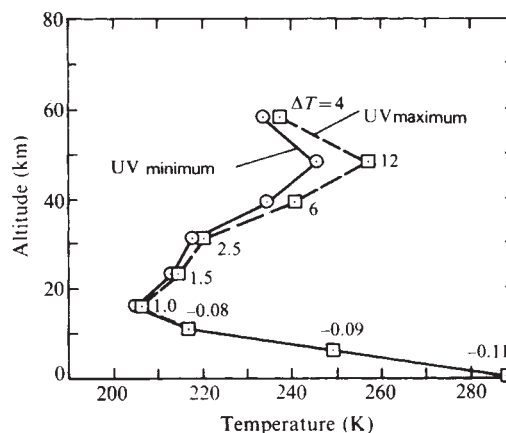


Fig. 1 Steady-state temperature profiles for times of UV maximum and minimum for long period perturbations (> 10 yr). Also shown are the temperature differences, ΔT , between these two cases.

situation by setting the fractional variation of UV irradiance equal to $C' \exp(-b'(T_\lambda - T_{\lambda_0})) \sin(2\pi t/P)$, in accord with limited observations over the 11-yr solar cycle^{1,2} where C' and b' are constants and T_λ and T_{λ_0} (4,750 K) are the brightness temperatures of the Sun at wavelengths λ and λ_0 , respectively.

The above alterations of the monochromatic solar fluxes were done only at wavelengths shorter than 0.29 μm , while the fluxes at longer wavelengths were altered uniformly in the opposite direction to hold the total luminosity constant. A value of 0.29 μm for the transition wavelength was selected as UV variations shortward of 0.29 μm have been reported^{1,2}, while severe limits have been placed on variations at visible wavelengths⁷.

We have carried out a series of calculations with the brightness temperature parameterisation of the spectral variations and with C' and b' chosen to generate UV variations consistent with the reported 11-yr variations² ($C' = 0.4$; $b' = 0.02$). For UV variations having periods > 1 yr, calculated peak-to-peak changes in ozone column density closely approach an asymptotic limit. Periods of 27 days and 6 months are characterised by peak-to-peak changes that equal about one-third and two-thirds of this asymptotic limit, respectively. Vertical transport limits the response of the system over these short time scales. We obtained very similar variations in ozone amount when the wavelength parameterisation was used and when the ratio of the amplitudes of the UV variation at 0.29 μm and 0.175 μm was varied by varying b' , but the amplitude at 0.175 μm (C') was held fixed.

The solar energy deposition profile is influenced by changes both in the trace gas constituents and in the solar spectrum. For all the driving periods considered, UV minimum is characterised by a smaller amount of energy absorbed in the stratosphere, a higher global albedo, and a larger energy deposition below the tropopause. Decreases in the ozone column density and the UV flux are responsible for these trends. Some of the sunlight not absorbed in the stratosphere during UV minimum is Rayleigh scattered back to space—hence the higher albedo—while the remainder is transmitted to the troposphere—hence higher deposition below the tropopause.

Figure 1 shows the radiative-convective equilibrium temperature profiles at times of UV minimum and maximum flux for the longest period cycles (> 10 yr). As might have been anticipated from the above discussion of solar energy deposition, the stratospheric temperatures increase from UV minimum to maximum, with the largest changes occurring near the stratopause. However, the tropospheric temperatures decrease, with the surface being about 0.1 K cooler during UV maximum.

Table 1 Predicted changes in atmospheric parameters due to solar spectral variations[¶]

Period	Epoch	Constraint* (refs)	ΔUV^*	ΔO_3^*	δT_{strat}^* (K)	δT_{surf}^* (K)	ΔS_{trop}^*
27 day	1969†	ΔUV (1)	0.05	0.003	0.1	-0.00003	-0.0002
6 month	1970†	ΔO_3 (19, 20)	0.15	0.013	2	-0.0006	-0.0005
11 yr	1966‡-70†	ΔUV (2)	1.5	0.13	12	-0.06	-0.005
11 yr	1970†-75‡	ΔUV (2)	1.5	0.13	12	-0.06	-0.005
11 yr	1966‡-70†	$\Delta O_3^ $ (19)	0.4	0.035	3	-0.02	-0.001
11 yr	1970†-75‡	$\Delta O_3^ $ (19)	0.4	0.03	3	-0.02	-0.001
22 yr	1958†-69†	$\Delta O_3^ $ (19)	0.8	0.07	6	-0.06	-0.003
Century	Late 17th§ late 18th centuries	$\delta T_{surf}^ $ (34)	3 #	0.26	23	-0.2	-0.01
Threshold values*		(refs 19-22)		±0.003	±0.5	±0.05	±0.01

* See text for definition.

† Close to sunspot maximum.

‡ Close to sunspot minimum.

§ Time of the Maunder sunspot minimum.

|| These values are upper bounds.

¶ In all cases, the ratio of the fractional change in irradiance at 0.175 μm to that at 0.29 μm has been set equal to 5, in accord with refs 1, 2.

We require a larger UV flux at the time of the Maunder sunspot minimum.

Evidently, the increase in downward directed thermal radiation from the stratosphere between UV minimum and maximum, due to the increase in ozone and the higher stratospheric temperatures, is less important for the tropospheric heat balance than the decrease in solar energy deposition below the tropopause. For the 11-yr period, the temperature changes in the troposphere will be only about half of their values for the longer period cases because the response time of the ocean-land-atmosphere system is comparable to the half period of the 11-yr solar cycle¹⁴. Correspondingly smaller responses characterise the shorter period cases. The computed changes in temperature structure are insensitive to the functional form chosen for the spectral variations, but they are sensitive to both the amplitude of the UV variation at 0.175 μm and the ratio of the amplitudes at 0.29 and 0.175 μm .

To assess the significance of changes in climate induced by solar spectral variations, we introduce the following parameters: δT_{strat} , δT_{surf} , which are the difference in stratopause (~48 km) temperature and surface temperature, respectively, between times of UV maximum and minimum, while ΔS_{trop} , ΔO_3 and ΔUV are the corresponding differences in solar energy deposited below the tropopause, ozone column density and solar UV intensity at 0.175 μm , respectively, divided by the values of these quantities at UV minimum. Table 1 summarises our estimates of these parameters for periods of spectral variation ranging from the 27-day solar rotation period to a century. In each case, an observed value of ΔUV , ΔO_3 , or δT_{surf} , as indicated by the 'constraint' column, has been used in conjunction with our model calculations to estimate the values of the remaining four parameters. Table 1 also shows our crude estimates of threshold values for the various parameters, below which a predicted variation would be small compared with known changes in its value. The threshold value is set at a quarter of the known change. The peak-to-peak variations associated with the quasi-biennial oscillation were used to derive the threshold values for δT_{strat} and ΔO_3 (refs 19, 20). Threshold values of ΔS_{trop} and δT_{surf} were obtained from a tidal theory for planetary waves²¹, and the globally averaged change in surface temperature over the past several decades²², respectively.

For changes occurring on time scales of weeks, good correlations have been obtained between solar activity indices and tropospheric circulation parameters²³. However, the reality of this solar-terrestrial relationship has been challenged because the above correlation is sensitive to which months are included in the analysis²⁴; no significant correlation is obtained for some epochs^{24,25}; and power spectral analyses of key meteorological variables fail to show a significant peak near 27 days²⁶. Since

solar UV variations may represent the most energetically important short-term modulation of solar output, it is of interest to estimate the impact of these variations on weather phenomena. As Table 1 shows measured values of ΔUV associated with the 27-day solar rotation are not expected to have caused significant alterations to any of the key atmospheric parameters, with the possible exception of a marginally significant alteration of the ozone column density.

For changes occurring on a somewhat longer time scale, a statistically significant correlation has been found between monthly averaged global ozone amount and solar activity indices^{27,28}. The maximum value of ΔO_3 was found to be 0.013, with a characteristic period of about 6 months. According to Table 1, about a threefold increase in ΔUV over the 27-day value is required to achieve this ΔO_3 . Even in this case, no significant alteration of the tropospheric parameters δT_{surf} and ΔS_{trop} results. However, small, but significant, changes occur in the stratospheric parameters δT_{strat} and ΔO_3 , which could conceivably induce marginal effects in the troposphere through stratospheric-tropospheric coupling processes²⁹. We conclude that, at best, UV spectral variations having time scales of weeks to months may occasionally perturb tropospheric weather in a barely noticeable manner.

From comparisons of measurements of solar UV irradiance made by similar broad band photometers carried on rockets and satellites, it has been suggested that the UV irradiance increased significantly from 1966 to 1970, with $\Delta UV \approx 1.5$ (ref. 2): 1966 was close to sunspot minimum and 1970 was close to sunspot maximum. Similar comparison of data obtained by satellite-borne spectrometers imply a decrease in UV irradiance between 1970 and 1975 of about the same magnitude². Some support for the validity of these estimates of ΔUV for the 11-yr sunspot cycle is given by an apparent agreement between observed ozone and temperature variations in the middle stratosphere during the late 1960s and early 1970s and predicted changes based on these ΔUV estimates³⁰. However, there are large error bars associated with this climate data set and it spans only about one sunspot cycle.

Consequently, we have examined additional data to assess further the accuracy of the above values of ΔUV for the 11-yr sunspot cycle. Estimates of the change in average ozone column density¹⁹ for the Northern Hemisphere that cover the actual times of the solar irradiance measurements imply $\Delta UV \approx 0.4$ and not 1.5 (Table 1). This disagreement is much larger than would be expected on the basis of the error bars assigned to the average ozone column densities. A slightly larger discrepancy results when the less well determined globally average ozone densities are used. Furthermore, the failure of the average ozone

column densities for the Northern Hemisphere and the world to increase from sunspot minimum in 1964 to sunspot maximum in 1958 raises some doubts as to whether the later variance (1964–75) is dominated by an 11-yr period. Also, much smaller changes in middle and upper stratospheric temperatures occurred over the time period of the first set of solar UV measurements (1966–70) than expected on the basis of the claimed UV variation and much smaller temperature changes occurred in the upper stratosphere over the interval of the second set^{20,31}. The ΔUV values given by Heath and Thekaekara² for the 11-yr solar cycle are, therefore, probably large overestimates of the true variation. According to Table 1, if all the variance in the average ozone column density between 1966 and 1970 and between 1970 and 1975 is ascribed to 11-yr UV variability, then significant changes in δT_{strat} and ΔO_3 result, but insignificant changes in δT_{surf} occur.

There seems to be a 22-yr periodicity in certain climate phenomena, such as the recurring drought on the high plains of the US³² and in other climate indicators³³. The time variation of average ozone densities between 1958 and 1976 may be dominated by a 22-yr period rather than an 11-yr period¹⁹. If all the variance in ozone amount between 1958 and 1969 is ascribed to 22-yr solar UV variation, then large changes in δT_{strat} result as well as a possibly significant variation in δT_{surf} . Conceivably, this value of δT_{surf} is large enough to account for the above cited 22-year climate changes.

Strong correlations have been shown to exist between century-long times of anomalous solar activity and climate anomalies, with periods of low solar activity corresponding to colder epochs^{3,4}. According to Table 1, if all the observed δT_{surf} for a period encompassing the Maunder sunspot minimum is ascribed to solar spectral variations, very sizeable changes in all the key variables result. It is reasonable to ascribe at least some fraction of δT_{surf} to this mechanism in view of the observed changes in the structure of the uppermost regions of the Sun associated with the Maunder minimum^{3,4}. Finally, aside from the significant temperature perturbations perhaps associated with longer term solar spectral variability, biologically significant changes in ozone amount also seem to be implied.

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Neogene explosive volcanicity, temperature and glaciation

J. R. Bray

PO Box 494, Nelson, New Zealand

Recent theoretical^{1–6} and observational^{7–10} demonstrations of depression in surface temperature of the Earth due to explosive volcanic eruptions have provided a more exact basis for speculations that explosive volcanicity may have had a climatic impact^{11–15} during periods over the past 2 Myr. In this letter, broad volcanic–climate relationships are assessed over a longer period by comparing the dates of global explosive phases and of volcanic–plutonic phases with paleotemperature and glaciation records from the Neogene. Analysis of these records indicates significant relationships between the explosive and volcanic–plutonic phases and Neogene temperature decline and also a correspondence between the three greatest explosive and volcanic–plutonic phases and glacial expansion. The second highest global explosive peak was coincident with recently suggested dates of around 3.2 Myr for the initiation of the late Cenozoic glacial age. Further testing of these relationships is necessary to resolve inconsistencies in the late-middle to early-late Miocene and in the late Pliocene.

To determine patterns of temperature trend for the Neogene, I examined six palaeotemperature curves^{16–21} for trend during geological epochs and their subdivisions and for trend on the absolute time scale of Berggren²². This examination indicated that the six studies were in agreement in showing two major periods of temperature decline, the middle Miocene and the late Pliocene or early Pleistocene with two studies^{16,17} also showing substantial declines, and another study¹⁸ a minor decline in the late Miocene. There was also predominant agreement on a temperature increase during the early Miocene and the middle Pliocene. Temperature trends, using Berggren's scale²², from Figs 5 and 6 of Kennett¹⁶, Fig. 16 of Margolis *et al.*¹⁷, Fig. 2 of Buchardt¹⁸ and Fig. 4 of Savin *et al.*¹⁹ are shown in Table 1. Results from the other two palaeotemperature studies^{20,21} were not included because they were less sensitive and were repetitive of the four presented curves. Table 1 also includes estimates of the occurrence of major glacial expansion periods which included dates of 16–14 Myr (ref. 16), 5–6 Myr (refs 16, 23) and 4.7–4.3 Myr (ref. 23) for Antarctica and 16 Myr (ref. 24) for the Northern Hemisphere.

The palaeotemperature and glaciation data in Table 1 were compared with two independent estimates of Neogene volcanic patterning. The first, by Kennett *et al.*²⁵, was based on a Pacific Basin marine ash survey which found four major Neogene explosive episodes that occurred (in descending magnitude) in the Quaternary (2–0 Myr), the middle Miocene (16–14 Myr), the latest Miocene to early Pliocene (6–3 Myr) and the late Miocene (11–8 yr). These patterns are similar to those of a global marine ash survey conducted by the same authors²⁵ and summarised in their Fig. 6. The second estimate of patterning of Neogene volcanicity was based on a global literature review which I made of 1,084 exactly dated volcanic and plutonic events, both terrestrial and marine. Examination of the distribution of these events suggested that there were two major and one minor Neogene volcanic–plutonic phases (number of volcanic–plutonic events per 10⁵ yr in parentheses) 3.3–0 Myr (17.7), 16.0–13.0 Myr (4.4) and 6.4–5.0 Myr (4.1) and three intervening periods of lesser volcanic–plutonic activity, 19.9–16.1 Myr (2.1), 12.9–6.5 Myr (2.7) and 4.9–3.4 Myr (3.6). Volcanic–plutonic activity peaks occurred at 19.5–19.4, 16.6–16.5, 15.7–15.6, 15.2–15.0, 14.6–14.5, 13.5–13.4, 11.4–11.3, 10.0–9.8, 8.3–8.2, 6.3–6.1, 5.8–5.7, 5.1–5.0, 3.1–3.0, 2.7–2.6, 2.4–2.3, 1.9–1.8, 1.2–1.0, 0.6–0.5 and 0.3–0.1 Myr.

Table 1 Neogene volcanic activity, glacial expansion and global palaeotemperature trends

Myr BP	Volcanic activity			Palaeotemperature			
	Explosive	Volcanic-plutonic	Glacial expansion	a	b	c	d
20-19				-	+	+	+
19-18				-	-	+	+
18-17				+	-	+	+
17-16				+	-	+	+
16-15	×	×	×	-	-	+	-
15-14	×	×	×	-	-	-	-
14-13		×		-	-	-	-
13-12				-	-	-	-
12-11				+	-	-	-
11-10	×			-	-	-	-
10-9	×			-	-	-	+
9-8	×			-	-	-	+
8-7				+	-	+	+
7-6				-	-	+	+
6-5	×	×	×	-	-	-	+
5-4	×		×	-	-	-	+
4-3	×			-	+	+	+
3-2		×		+	-	-	-
2-1	×	×	×	+	-	-	-
1-0	×	×	×	-	-	-	-

Explosive episodes from Kennett *et al.*²⁵; volcanic-plutonic episodes from global literature survey. Glacial expansion periods as in text. Palaeotemperature trends: a, Kennett¹⁶; b, Margolis *et al.*¹⁷; c, Buchardt¹⁸; d, Savin *et al.*¹⁹. Plus indicates an increasing, and minus a decreasing global temperature.

The geological occurrence of the four explosive phases in the Quaternary, middle Miocene, latest Miocene to early Pliocene and the late Miocene, as described by Kennett *et al.*²⁵, overlapped the three main global temperature decline periods outlined above which occurred in the late Pliocene to early Pleistocene, middle Miocene and the late Miocene. During the two greatest explosive episodes, from 16-14 and 2-0 Myr, 14 of 16 temperature estimates showed a decline. For the moderate explosive episodes of 11-8 and 6-3 Myr, there were 17 of 24 temperature estimates that declined. In the remaining, non-explosive intervals, 21 of 40 temperature estimates showed a decline. To test the validity of these patterns, the temperature data in Table 1 were compared with the four Kennett *et al.* explosive episodes, using a fourfold contingency table. The result gave a χ^2 of 5.5 ($P < 0.02$) which suggests a connection between the occurrence of Neogene explosive episodes and a tendency towards temperature decline. A fourfold contingency χ^2 test was also applied to the volcanic phases which occurred at 16-13, 6-5 and 3-0 Myr in the global volcanic-plutonic survey and the temperature data in Table 1. The result gave a χ^2 of 8.1 ($P < 0.005$), again suggesting a Neogene volcanism-temperature relationship.

Examination of the possible volcanism-temperature relationship in Table 1 indicates an anomaly in the late Middle to early late Miocene around 14-11 Myr during which time 11 out of 12 palaeotemperature data showed a decline during a period of low explosive activity. Data in the global survey of Kennett *et al.*²⁵ indicate, however, that the level of ash produced in this period was only moderately less than in the preceding and succeeding explosive episodes which suggests that the trend of temperature decline which began during the early middle Miocene may have been sustained by the moderate activity from 14 to 11 Myr.

There was a correspondence in Table 1 between the major Neogene glacial expansion periods at 16-14, 6-4 and 2-0 Myr and both the three highest Neogene explosive episodes and the three volcanic-plutonic phases. The apparent lack of a major glacial expansion at the explosive episode around 11-8 Myr could relate to the explosive level during this interval which had

the lowest activity of the four Neogene episodes designated by Kennett *et al.*²⁵.

The second highest Neogene explosive peak in Kennett *et al.*²⁵ (Fig. 2) was from 3.5 to 3.0 Myr. Kennett *et al.*²⁵ in noting this secondary explosive peak stated that there were also many lavas of this age in the circum-Pacific, but that there were not enough data to determine whether this 3.5-3.0 Myr peak was distinct from, or part of, the explosive episode from 6 to 3 Myr. The peak around 3.5-3.0 Myr is also present in a global survey of volcanic-plutonic activity which I have made and in local explosive studies including a well-dated upswing which began between 3.2 and 3.1 Myr in Japan²⁶. This major explosive peak from around 3.5-3.0 Myr was concurrent with an extensive and globally well-distributed array of estimates of major temperature decline around 3.2-3.1 Myr (refs 26-30) and glacial expansion around 3.5-3.1 Myr (refs 31-35) which are currently regarded²⁸ as the date for the initiation of the late Cenozoic glacial age.

From around 3.0 Myr to the present, the global explosive data²⁵ show a period of low output from 3.0 to 2.0 Myr and then a maximum activity from 2.0 to 0 Myr. As the climatic data suggest that there was a period of cooler temperatures and cyclic glacial expansions from around 3.2 to the present²⁸, the low global volcanic record from 3.0 to 2.0 Myr may contradict the volcanic climatic hypothesis. Regional studies³⁶⁻³⁸ from ocean cores in the North Pacific indicate, however, that there was a generally high level of explosive activity during the 3.0-2.0 Myr interval with a major pulse around 2.5-2.3 Myr. Further and more sensitive dating of the global explosive data may resolve this inconsistency.

The Neogene volcanism-climate relationships shown above were assessed under the assumption that it is the total level of explosive activity that may have had a climatic impact. It is conceivable, however, that a relatively few single or closely spaced massive eruptions could have acted as trigger devices³⁹ for the 'instantaneous glaciation' mechanism and that it is the occurrence of these massive eruptions which is climatically significant. This possibility is not contradicted by the demonstrated correspondence between the Neogene explosive episodes, when massive eruptions more likely occurred, and the climatic parameters in Table 1.

Since writing the above, I received a manuscript from Kennett⁴⁰ which has conclusions broadly similar to those presented here, and includes the statement that the four explosive phases outlined in Kennett *et al.*²⁵ coincide with "critical developments in the climatic and glacial evolution of the earth".

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India's and Australia's pole path since the late Mesozoic and the India–Asia collision

Chris T. Klootwijk

Research School of Earth Sciences, Australian National University,
PO Box 4, Canberra ACT 2600, Australia

John W. Peirce

Petro Canada, PO Box 2844, Calgary, Alberta, Canada

The Indian apparent polar wandering path (APW) for the late Mesozoic–Cenozoic period has been too poorly defined to use as a constraint to establish the timing of collision of India and Asia. Here we update the Indian APW on the basis of new palaeomagnetic data from DSDP cores and from Baluchistan (Pakistan). This new synthesis details the late Mesozoic and Cenozoic northward motion of India, and supports geological evidence for an initial collision with Asia during the late Palaeocene–early Eocene.

Recent attempts to trace India's northward motion and collision with Asia have used two approaches. Seafloor spreading data from the Atlantic and Indian Oceans were used to detail the relative Indian–Eurasian motion¹. According to this rather generalised approach intimate contact between the two continental masses was established by Eocene–Oligocene time (~40 Myr), based on a reduction in the rate of relative motion from an initial 10 to 16 cm yr⁻¹ to about 5 cm yr⁻¹.

On the other hand, using the relative motion data of the Indian, Australian and Antarctic plates^{2–4} to reconstruct the Indian–Australian relative motion and the Australian APW^{5–7} for positioning the Indian plate relative to the pole, others have argued^{8,10} that initial collision occurred during late Palaeocene–early Eocene time (~55 Myr; ref. 9). India's northward motion reduced at that time from 15–20 cm yr⁻¹ to <4–6 cm yr⁻¹, which supports earlier advanced geological arguments¹⁰ for a pre-Middle Eocene timing of collision.

The DSDP pole positions¹¹ were obtained as 10 Myr averages of individual pole positions, determined from (1) mean palaeolatitudes at a given site and at a given age corrected for a systematic downwards deflection due to absence of declination control^{12,13} and from (2) declinations at a given site and at a given age simulated¹¹ from seafloor spreading data as summarised by Powell *et al.*¹⁴ The Baluchistan data in part come from an allochthonous structural unit, but results have been corrected for a well determinable clockwise rotation¹⁵.

This new Indian APW (Fig. 1) facilitates direct tracing of India's northward movement (Fig. 2a). New palaeomagnetic data from Ladakh (north-west Himalaya¹⁶), north of the Indus–Tsangpo suture zone, have established the palaeoposition of the Ladakh segment of the Kohistan–Ladakh island arc (Fig. 2b, refs 17, 18) before collision and during cooling (K–Ar ages 45–49 Myr, see ref. 16) after its collision with northward-moving continental Indo–Pakistan. Comparison of Fig. 2a with 2b evidently supports the postulated pre-Middle Eocene data of

collision^{8,10}. On the basis of geological evidence from north-western Indo–Pakistan this postulation can now be refined as initial collision between continental Indo–Pakistan and an island arc off southern Asia, which occurred most probably during the late Palaeocene–early Eocene^{9,11,16,19,20}.

The former southern boundary of Asia, juxtaposed to India, is not well defined, but can be taken as the eastwards continuation of the Zagros–Makran convergence zone⁹. The 2–10°N palaeolatitude of the Ladakh segment of the Kohistan–Ladakh island arc before and on initial collision, is far to the south of the contemporaneous position of this extrapolated Zagros–Makran convergence zone relocated according to Eurasian palaeomagnetic data²¹. Closure of a hypothesised marginal basin along the further northwards positioned Shyok zone¹⁷ can possibly be related to a reduction in the rate of the Indian–Eurasian relative movement at the Eocene–Oligocene time boundary as interpreted elsewhere^{1,12}.

Relocation of India according to Australian palaeomagnetic data and plate relative movement, disagrees (but just not significantly at the 95% level) for at least the early Tertiary period (Fig. 2c) with direct palaeolatitudinal observations from the Indian plate (Fig. 2a) and from Ladakh (Fig. 2b). India's indirectly obtained palaeoposition according to the Australian Barrington Volcano result (Fig. 2c; 52 Myr) is about 1,000 km further north than its directly observed position at the time of initial collision. The DSDP core data from the Indian plate and the results from Ladakh are in mutual agreement for the early Tertiary. Therefore, the above discrepancy has to be explained either by an error in the relative-motion data or by an inaccuracy in the Australian APW. The scale of the discrepancy seems beyond that which it is reasonable to expect in the relative motion data. One of us (J.W.P.)¹² previously indicated that an age of the Barrington Volcano about 10 Myr younger than the determined 51.7±0.7 Myr (ref. 5) would resolve the discrepancy. K–Ar ages as young as 43 Myr were reported for this volcano, but were not considered to have significant bearing on the age of the palaeomagnetically studied flows (ref. 5 and I. McDougall, personal communication). Therefore, the dis-

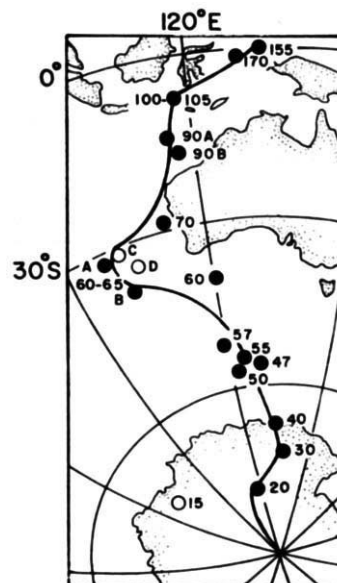


Fig. 1 APW for India based on data from continental Indo–Pakistan and DSDP core data from the Indian plate (Table 1). The ages indicated represent radiometric ages or the mean of stratigraphic age ranges as used in the determination of transferred pole positions (Table 1, Fig. 3a). ○ The Murree–Siwalik pole position²² from the allochthonous Salt Range–Potwar Plateau structural unit, and the pole positions from the Lower and Upper Deccan Trap successions (60–65 C and D respectively) determined from maximum grouping of a density distribution of individual poles¹¹ as alternatives to Fisherian mean pole positions (60–65 A and B, respectively). Linear polar projection.

Table 1 Late Mesozoic and Cenozoic palaeomagnetic south pole positions for India and Australia

Formation	Age* (Myr)	Original†			Transferred		Ref.	
		Lat (°S)	Long (°E)	α 95 (°)‡	Lat (°S)	Long (°E)		
A1) Australia, also transferred to India								
Cygnat alkaline complex	98 (98)	53	156	10	14	124	23	
Mt Dromedary complex	93 (93)A	56	138	9	12	114	24	
Mt Dromedary complex	93 (93)B	56	153	10	16	121	25	
Bunbury basalt	>90–100 (90)	49	161	10	14	131	25	
Morney profile	Maastrichtian–Lower Eocene (57)	60	118	4	43	109	26	
Older volcanics Victoria	Paleocene–Eocene? (55)	61	144	17	53	133	27	
Barrington volcano	51.7 (51.7)	70	126	5	65	123	5	
Liverpool volcano	33.7 (33.7)	71	96	5	70	98	5	
Canaway profile	Upper Oligocene? (28)	74	115	7	73	117	26	
Lavas and Dykes, SE Queensland	20–22 (22)	78	86	12	78	90	24	
Tweed volcano	20–22 (21)	72	121	5	71	121	28	
Main range volcanics	20–22 (21)	77	98	7	76	100	28	
Nandewar volcano	17–18 (17.5)	78	84	5	78	87	5	
Newer volcanics Victoria	0–5 (2.5)	87	86	2	87	87	29	
A2) Mean Cenozoic results for Australia, also transferred to India								
	95 (95)	55	147	7	13	119	30	
	40–60 (50)	69	131	5	64	127	6,7	
	35–25 (30)	69	92	4	68	94	6,7	
	25–20 (22.5)	77	111	4	77	113	6,7	
	20–15 (17.5)	79	66	4	79	69	6,7	
B1) India, also transferred to Australia								
Loralai limestone§	middle Jurassic (170)	2	128	8	34	183	15	
Chiltan limestone	Oxfordian to Lower Kimmeridgian (155)	1	132	10	31	184	15	
Rajmahal traps	100–105 (102.5)	8	117	4	34	151	11	
Goru Fm-Parh Lst Sanjawi§	Aptian–Albian to Santonian–Campanian (90)	18	117	3	57	160	15	
Goru Fm-Parh Lst Quetta	Aptian–Albian to Santonian–Campanian (90)	15	115	4	55	155	15	
Lower reversed Deccan traps	60–65 (63.7)A	33	98	4	51	108	11	
Lower reversed Deccan traps	60–65 (63.7)C	32	101	—	50	113	11	
Upper normal Deccan traps	60–65 (61.2)B	39	101	5	54	111	11	
Upper normal Deccan traps	60–65 (61.2)D	35	104	—	50	114	11	
Brewery limestone	base Upper Palaeocene (57)	53	118	22	63	138	15	
Sanjawi limestone§	Palaeocene–Eocene (55)	55	123	15	63	129	15	
Zam limestone§	Middle Eocene (47)	57	129	14	61	131	15	
Murree–Siwaliks¶	Miocene (15)	72	69	17	72	69	22	
B2) DSDP core data Indian Plate, also transferred to Australia								
	65–75 (70)	29	111	—	56	133	11–13	
	55–65 (60)	41	120	11	54	133	11–13	
	45–55 (50)	58	121	9	62	123	11–13	
	35–45 (40)	67	132	12	72	136	11–13	
	25–35 (30)	72	136	4	74	136	11–13	
	5–22.5 (20)	79	115	—	79	113	11–13	

* Stratigraphic or K–Ar ages. Mean ages used in transferring the palaeomagnetic pole position are shown in parentheses.

† Finite rotations for the Australia–Antarctica motion were according to the summary in ref. 31 for the period up to 45 Myr BP, and according to ref. 32 for reconstruction of the initial fit.

Myr BP	Lat (°N)	Long (°E)	Angle (°)
10	9.7	36.5	–6.75
21	13.8	34.6	–12.0
29	14.25	33.25	–16.0
35	12.5	34.4	–18.9
45	10.3	34.75	–23.6
> 50	11.9	30.8	–30.9

Eulerian rotation poles for the India–Antarctica motions were according to the summary in ref. 3 (fixed to Antarctica).

Myr BP	Lat (°N)	Long (°E)	Angle (°)
0–32	4.9	36.4	18.3
32–53	–16.0	27.4	18.4
53–64	4.0	–3.0	15.6
64–80	–2.0	8.0	25.24
80–130	12.29	144.20	–21.85

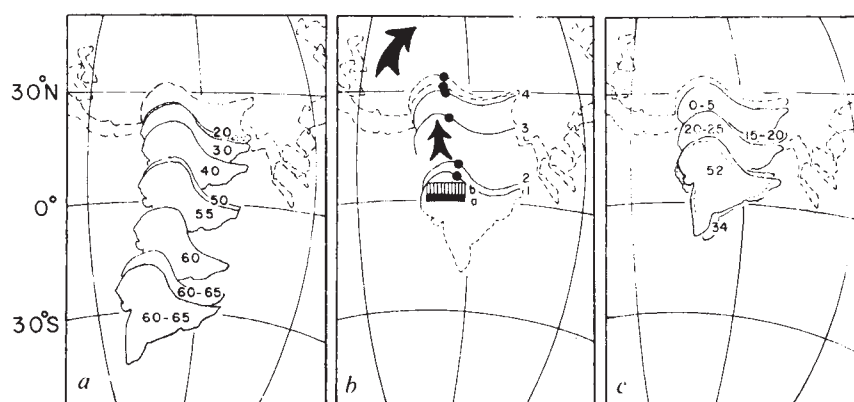
‡ The errors (α_{95}) refer to the 95% confidence limits of the original pole position only, and do not include estimates of the errors in the finite rotations.

§ Results corrected for an inferred clockwise rotation of the Sulaiman Lobe over 50°.

^{||} Mean result obtained from density distribution of palaeomagnetic pole positions¹¹.

¶ The Salt Range–Potwar Plateau Unit is most probably an allochthon, detached from basement¹¹.

Fig. 2 Successive palaeolatitudinal positions of India according to pole positions from the Indian plate (Fig. 2a) and transferred pole positions from Australia (Fig. 2c). Note the discrepancy between India's palaeoposition according to the transferred Barrington Volcano pole position (Fig. 2c; 52) and the palaeopositions according to Indian data of comparable age (Fig. 2a; 50, 55). India's northern boundary is drawn along the Indus-Tsangpo suture zone and its western and eastern continuation. Figure 2b shows the palaeolatitudinal position of the Ladakh segment (large dots) of the Kohistan-Ladakh island arc north of the Indus-Tsangpo suture zone, according to four magnetisation components determined¹⁶ in the Ladakh Intrusives (1 and 2: ~50 Myr BP and postdating initial collision of continental Indo-Pakistan with the island arc; 3 and 4: younger magnetisation components probably resulting from younger phases of the Himalayan orogenesis). For enhanced visibility the Indus-Tsangpo suture zone is shown in its present form and position relative to the Ladakh region. Zone a indicates the palaeolatitudinal position of the Upper Cretaceous Dras Flyschoids outcropping within the suture zone¹¹, and zone b indicates the palaeolatitudinal position of the Ladakh region according to the sampled lower Tertiary to possibly Upper Cretaceous Indus Molasse which overlies the Ladakh Intrusives¹¹. The present outline of southern Asia is positioned according to mean palaeomagnetic results from Eurasia²¹. a: --- 60 Myr; --- 20 Myr. b: --- 50 Myr; --- 20 Myr. c: --- 50 Myr; --- present day. Linear polar projection.



crepancy probably results from inaccuracy in the pole position of the Barrington Volcano.

For a further check on discrepancies between the Indian and the Australian APWs, late Cretaceous and Tertiary pole positions from the Indian plate have been transferred to the Australian plate and the alternative APW for Australia thus obtained is shown in Fig. 3a. In Fig. 3b, this indirectly obtained APW is compared with the hitherto established late Cretaceous-Tertiary APW for Australia, as compiled before from all previously available Australian palaeomagnetic data^{6,7}. This zig-zag shaped direct APW for Australia (dashed line connecting squares) is either notably displaced from the indirectly obtained APW (Fig. 3b, ■ 30), does not agree with it in position or age (Fig. 3b, ■ 50), or is not sufficiently detailed to show the pronounced 60–65 Myr kink which is present in the indirect APW. These observed discrepancies reflect possible inaccuracies in the pole positions of respectively the Barrington Volcano (Fig. 3b, ● 52) and the Liverpool Volcano (Fig. 3b, ● 34). Apart from these discrepancies most other Tertiary pole positions for Australia (Fig. 3b, ●s) follow the indirect APW at an only slightly westwards displaced position.

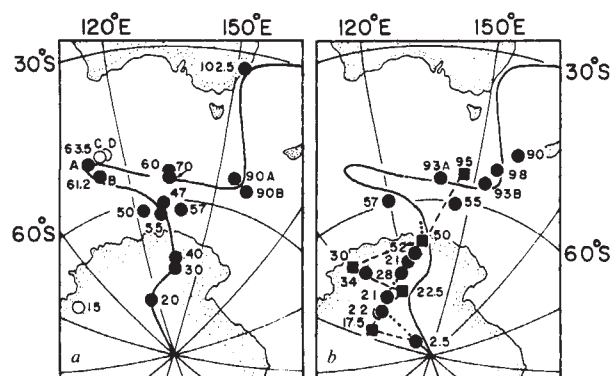


Fig. 3 a, The Indian APW (full line) and Indian pole positions (● and ○) transferred to Australia. b, Comparison of the indirectly obtained APW for Australia with an earlier compiled interpretation of Australia's APW (refs 6, 7; dashed line) and with individual Australian pole positions (●). Compare with Fig. 1. See text for discussion and Table 1 for pole positions and confidence limits. The latter are not shown in the figures to preserve visibility. Linear polar projection. The circle (a, 15) indicates the transferred Indian Murree-Siwalik pole position²². Its quoted support⁷ for the zig-zag shaped Australian APW does not seem warranted as this ill-constrained result comes from the allochthonous Salt Range-Potwar Plateau structural unit, whose rotational movement is not fully determined¹¹.

The indirectly obtained APW is proposed here as an alternative to the directly observed APW for Australia. The former must be considered speculative until substantiated by further (transferred) Tertiary palaeomagnetic data from continental Indo-Pakistan and corroborated by forthcoming data from Australia. Note that the zig-zag shaped direct APW for Australia is up for refinement and that previous relocations of India based on this APW can no longer be considered to be accurate. India must be positioned according to palaeomagnetic data from the Indian plate.

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An Archaean suture in Sierra Leone?

H. R. Williams

Department of Geology, University of Leicester, UK

Considerable evidence has appeared supporting plate-tectonic mechanisms for the production of Archaean metamorphic terrains¹⁻⁴. Windley¹ has proposed models to explain both the relationship between the high grade 'gneiss' and the low grade 'granite-greenstone' terrains and the building of continental crust by collision of small continental masses. His model uses the same geological mechanisms as those typically exhibited by Phanerozoic orogenic belts, except that they were more rapid during the Archaean as a result of the fast seafloor spreading rate at that time^{5,6}. I propose here that an extensive zone of mylonites, up to 10 km thick, on the coastal margin of the West African Craton^{7,8} is a suture developed during the late Archaean at the site of collision of two granitic continental masses, now preserved in a partially reworked state as the Guyana Shield and the West African Craton (Fig. 1). The suture is evidence for continental coalescence in the late Archaean.

In the vicinity of Sierra Leone the mylonite is at least 400 km long and consists of severely tectonised gneisses and mylonites nearly 10 km thick⁷. The mylonite is broadly divisible into a 5-km thick zone of fine grained mylonites of both basic and granitic composition, underlain by up to 5 km of flaggy gneisses of variable composition. The gneisses and mylonites are well banded, contain deformed and granulated augen and exhibit ribbon texture. Tectonic interleaving of the rocks on each side of the mylonite zone occurred at an early stage resulting in mixed basic and granitic gneisses and mylonites. This zone of extreme deformation is sometimes not as fully developed as suggested; in southern Sierra Leone and western Liberia, for example, the mylonites seem to be poorly developed or absent.

Structurally above and to the west of the mylonite zone is a granulite facies supracrustal belt, known as the Kasila Group^{7,8}, which forms a linear north-west-south-east trending intensely-deformed zone adjacent to the Liberian granite-greenstone terrain of the West African Craton (Fig. 1). Approximately 8 km of fine grained basic granulites containing subordinate banded iron formation, semi-pelites and quartzites are overlain by 5 km of amphibolite, metasedimentary granulites and migmatites. The lower part of the southwesterly dipping succession is characterised by the presence of sills of coarse-grained layered leucogabbro⁷.

Structurally below and to the east of the mylonite zone is the extensive granite-greenstone terrain of the West African Craton, which typically yields radiometric ages of 2,700 Myr⁹. Consisting predominantly of granodioritic gneisses and migmatites, the terrain has a structural trend discordant with the adjacent Kasila Group and mylonite zone, being generally north-south as defined by the thin synclinal strips of amphibolite facies supracrustals.

The thickness and longitudinal extent and the structural and metamorphic characters of the Kasila Group strongly contrast with the supracrustal greenstones of the adjacent terrain. The Archaean gneisses and granulites of the Guyana Shield, although partly reworked as a result of early Proterozoic activity^{9,15} tend to be of variable structural trend and like the granite-greenstone terrain of the West African Craton contrast with the essentially linear structure and extreme metamorphic grade of the Kasila Group. Although the Kasila Group is regionally discordant with the greenstone trends in the adjacent terrain, the granitic terrain immediately adjacent to it is deformed into parallelism with the high grade belt^{10,11}.

Some 20 km to the east of the mylonite zone is a series of disconnected exposures of low grade recumbently folded Archaean supracrustals, known as the Marampa Group⁷, with a lower contact thrust against the underlying granitic rocks. They

are clearly not part of a greenstone belt structure and being allochthonous, were perhaps derived as a nappe sheet from an upper level of the adjacent Kasila Group before its extreme deformation and metamorphism.

The presence of the mylonitic thrust zone, the Marampa Group klippen, the strong contrasts in stratigraphy, structural and metamorphic styles between the Kasila Group and the adjacent terrain suggests that the two major structural units were brought together tectonically. One mechanism for the production of the observed field relations could be that of a continental collision suturing orogeny similar to that envisaged for the Dalslandian^{12,13}, or for the Himalayas¹⁴. The Kasila Group supracrustals might originally have been deposited in an intracratonic or small ocean basin between two recently stabilised granitic terrains which were then driven together through the active plate-tectonic regime that characterised the late Archaean^{2,5,6}. The act of complete suturing could trap basaltic crust and supracrustals between the two masses, squeezing up some at an early stage to form the Marampa Group nappe sequence, while the remainder became steadily attenuated in a vice-like situation (Fig. 1). Closure was accompanied first by plastic deformation in granulite facies conditions and then brittle deformation in retrogressive metamorphic conditions, giving rise to a thick sequence of fine grained granulite facies rocks, underlain by thrust zones of flinty material, underlain in turn by porphyroblastic flaggy gneisses. Post-collisional deformation might have thickened the zone of suturing by shortening and duplication, during which time the Kasila Group and the immediately adjacent crust were subjected to a high-temperature metamorphism due to burial in the locally thickened crust. Subsequent isostatic rebound allowed erosional processes to unroof the suture zone as a mass of material which is of a higher grade of metamorphism than the surrounding terrain.

On a much larger scale and at a deeper level of erosion, these field relations are similar to those of the root zone to the Helvetic nappes in the Ursuren Zone between the Aar and Gotthard Massifs in the Swiss Alps. The Ursuren Zone consists of a steeply dipping body of supracrustals bounded on both sides by zones of extreme deformation in which both supracrustals and the enclosing granitic material have been subjected to a very intense shear deformation. The later stages of the Alpine orogeny apparently forced the subduction of the granitic Aar massif below that of the Gotthard massif, while the sediments that were trapped between the two became intensely deformed in the

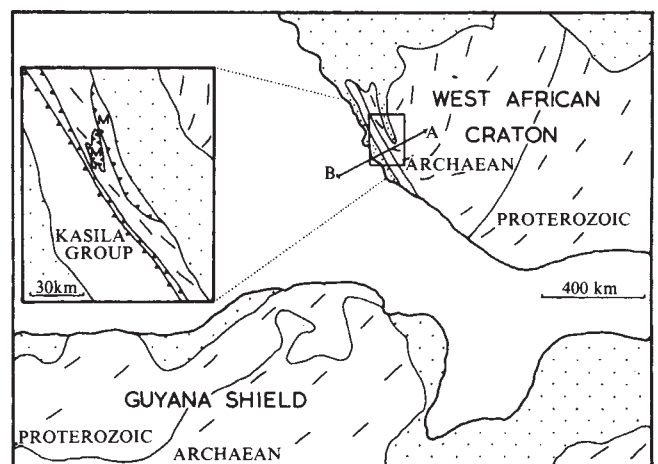


Fig. 1 Reconstruction of pre-mesozoic geology of the West African Craton and the Guyana Shield. Trend lines indicate regional structure of Archaean, much of which is partially reworked during the early Proterozoic. Post early Proterozoic cover indicated by stippling. Line A-B indicates position of idealised cross-section shown in Fig. 2. Inset shows detail of geology of western Sierra Leone, indicating the spatial relationships between the Kasila Group, the Marampa Group (M), and the thrusts and mylonites of the suture zone (thrust symbols).

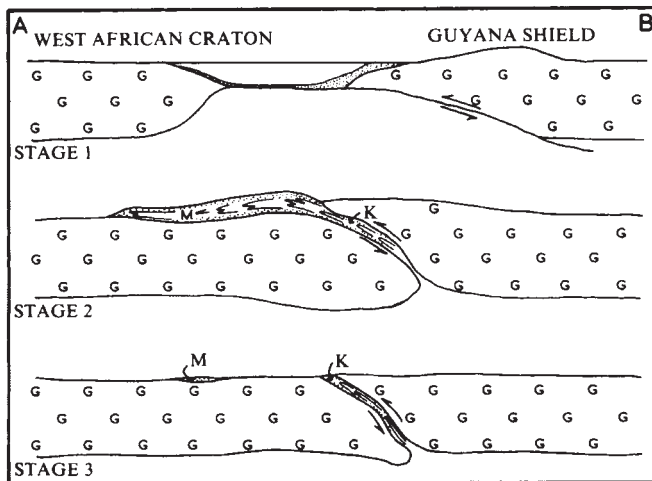


Fig. 2 Cartoon of envisaged sequence of events leading up to the formation of the Archaean suture. Stage 1, convergence of continental plates, closing of sedimentary basin floored by basaltic material. Stage 2, collision, causing plastic deformation of the Kasila Group to produce nappe sequence at high level, with granulite facies shear zones at deep level. Thickening and uprise of suture zone, overriding of the Guyana Shield onto the West African Craton. Stage 3, continued over-riding, brittle thrusting, mylonite formation in retrogressive metamorphic conditions, rapid erosion with isolation of Marampa Group as klippen.

suture zone. The granitic material on either side of the suture was also deformed, ultimately becoming schistose. If allowance has been made for the difference in scale there are strong similarities between the Cenozoic Alpine and Archaean West African examples of a collisional suture.

The collision along the mylonite zone also explains the strongly developed brittle fractures in the adjacent Archaean granite-greenstone terrain of Sierra Leone^{7,11}. Two main directions of fractures are obvious from the regional drainage pattern, forming a conjugate pair, one trending roughly north-south, the other east-north-east. Ideally, these two directions of fractures could be produced by a compressive stress acting from the south-west during the late Archaean suturing orogeny.

Post-Palaeozoic seafloor spreading has separated the cratonic masses which united so violently during the late Archaean, but has done so close to the line of the original suture. Kennedy¹⁶ noticed that Pan African tectono-thermal belts tended to act as the loci for subsequent rift structures in the Mesozoic. As a result, sutures formed in early orogenic belts are susceptible to later plate activity, frequently becoming destroyed as plate-margins in orogenic belts.

If the mylonite zone and the associated high grade Kasila Group supracrustals represent a suture developed as a result of two continental masses colliding at the end of the Archaean, then it supports the opinion that primitive plate tectonic mechanisms were active in the Archaean.

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Sex ratio and niche differentiation in spinach (*Spinacia oleracea* L.)

S. S. Onyekwelu* & John L. Harper

School of Plant Biology, University College of North Wales, Bangor, UK

Studies on the population biology of dioecious plants have concentrated on deviations from the expected sex ratio of 1:1 (refs 1-4) and on sexual dimorphism⁵⁻¹⁰. Sexual dimorphism may imply that the two sexes have different ecologies and this has been demonstrated for a number of animals¹¹. Clearly, if males and females occupy at least partially different niches, competition between members of the two sexes will be reduced and observed sex ratios may be biased by the relative carrying capacities of the two niches. Putwain and Harper¹² described differences in the seasonal patterns of growth of males and females in *Rumex acetosa* and *Rumex acetosella* and Freeman *et al.*¹³ showed that the males of five dioecious plant species in northern Utah were more abundant on xeric microsites, while females were over-represented in the moister regions of each local environment. We report here that the growth of male and female plants of the annual *Spinacia oleracea* L. var. Viking is affected differently by population density, and this is reflected in the sex ratio.

The experiment was carried out in the field at Pen-y-ffridd Experimental Station at Bangor, North Wales. Seed was sown on 6 June 1978 at three densities—120, 600 and 3,000 seeds per plot, 50 × 50 cm (equivalent to distances between the seeds of about 5 cm, 2 cm and 0.9 cm, respectively). There were four replicate plots at each density in a fully randomised design.

Male plants started to flower on 22 July and the first female plants did not flower until 2 weeks later. During this 2-week period the males were very conspicuous and a casual observer could easily have recorded the population as wholly composed of males. The apparent sex ratio changed considerably after 6 August as females began to flower and branch vigorously. The populations were harvested on 17 August, when several plants had still not flowered and were spindly and trailing beneath the canopy of the flowering plants. It is unlikely that any of them would have flowered if left for longer. At harvest the male, female and unflowered plants were counted. The mean percentage dead leaves per plant (a measure of the extent of senescence) and the mean number of axillary shoots per plant were determined separately for male and female plants. The number of dead male and female plants was also recorded. Table 1 shows the proportions of sown seed that formed plants present at harvest, the proportions of plants that flowered and the sex ratios of the flowering plants. The populations showed three distinct density-dependent reactions. With increasing density the proportion of seeds giving plants still present at harvest fell from 0.546 to 0.143. Of the plants that survived to harvest the proportion failing to flower increased from 0.195% to 0.318% with increasing density. Males formed an increasing fraction of the total flowering plants as density increased. At the lowest density the sex ratio did not deviate significantly from 1:1 but at medium and high densities there was significant deviation in favour of male plants.

There is no reason to doubt that the sex ratio was equal among the seeds sown. Of the plants that were present at harvest a sufficient number had remained vegetative or had died to account for all the deviations in the observed sex ratios.

The mean dry weight and the mean number of axillary shoots borne by females at harvest was significantly greater than that of the males at all densities (Tables 2 and 3), reflecting their

* Present address: Department of Botany, University of Nigeria, Nsukka, Nigeria.

Table 1 Survivorship, flowering and sex ratios in populations of *Spinacia oleracea* sown at three densities

Density	Total	Total as % of seeds sown (that is, survivorship)	Failed to flower	Failed to flower as % of plants surviving	Flowered		Flowered ♂ & ♀ as % of all flowering plants		χ^2 for deviation from expected 1:1 ratio of males to females		P
					♂	♀	♂	♀	Heterogeneity (d.f. = 3)	Pooled (d.f. = 1)	
Low	262	54.6%	51	19.5%	100	111	47.4%	52.6%	1.387	0.573	NS
Medium	903	37.6%	240	26.6%	393	270	59.3%	40.7%	7.584	22.89	$P < 0.001$
High	1,716	14.3%	545	31.8%	729	442	62.3%	37.7%	4.177	70.34	$P < 0.001$

Table 2 Mean dry weight per plant of flowering male and female plants of *Spinacia oleracea* in populations sown at three densities

Density	Mean (g)		s.e. of difference between the two means	Degree of freedom
	♂	♀		
Low	1.50	2.31	0.454	6
Medium	0.73	1.55	0.1612*	6
High	0.50	0.88	0.1183†	6

Significance levels: *0.01; †0.05.

Table 3 Mean number of axillary shoots developed in flowering male and female plants of *Spinacia oleracea* in populations sown at three densities

Density	Mean (g)		s.e. of difference between the two means	Degree of freedom
	♂	♀		
Low	3.92	6.66	0.3730*	6
Medium	2.27	5.43	0.3252*	6
High	1.32	4.56	0.4827*	6

Significance level: *0.001.

continued growth, in contrast to many of the early flowering males which were already dead at the time of harvest (Table 4). The earlier senescence of the males is shown by the proportion of dead leaves borne by the plants which was higher on male plants at all densities (Table 5).

These results show both sexual dimorphism and niche separation in time in the development of the two sexes in spinach. Males are precocious in growth and flowering time but tend to senesce and die earlier than the females which continue to grow and develop axillary branches. While the earlier flowering males are dying, most of the females are setting seeds and continuing to increase the area of leaf both on the main stem and on the new axillary shoots. The continued growth of the females may be made possible by resources (for example, light) freed by the death of male plants.

Precocious flowering and senescence of the males must imply considerable wastage of pollen. However, the early death of male plants at the time when the females are setting seed may increase the reproductive vigour of the female plants. The evolution of this type of sexual niche differentiation might be explained if the dispersal of seed and pollen is highly localised so that a male is likely to favour his own progeny by dying early.

Niche separation of sexes in space or in time is probably one of the causes of observed unbalanced sex ratios in dioecious plants. In a population where one sex flowers and dies earlier than the other, the time of observation of the sex ratio becomes critical and in the present study the observed male sex ratio changed considerably over 6 weeks. A number of deviant sex ratios have been reported in *Spinacia oleracea*. Zwaan¹⁴ found that spinach sown in a hot and dry climate produced an excess of male plants while seed sown in the cool moist climate of Holland produced nearly equal numbers of males and females. Rosa⁶ commented, however, that such tests of 'place effect' were not reliable unless the different plantings came from the same seed samples. Hoffman had observed that the sex ratios of spinach appeared to vary according to the spacing of the plants (quoted in ref. 15).

When plants were crowded or more or less stunted, males predominated, but with wider spacing and better growing conditions there were relatively more females (he did not give figures). When the two sexes are ecologically different and interacting competitively it is not surprising that sex ratios are altered by changes in density. Rosa⁶ grew six varieties of spinach in different conditions of nutrition, spacing, shading, date of planting and mutilation. He concluded that none of these factors had any appreciable influence on sex expression but he used a lower range of densities (interplant distances of 15, 5 and 2 cm) than in the experiment reported here.

In view of the sexual niche differentiation (the Jack Sprat effect) described here for *Spinacia*, for *Rumex* species by Putwain and Harper¹² and by Freeman *et al.*¹³ it is pertinent to ask how large a role such ecological differentiation may have played in the evolution and maintenance of dioecy.

There is no reason to propose that the resource demands made in the production of male gametes are the same as those involved in producing female gametes and nurturing embryos. Certainly the time sequence of resource consumption in producing male and female gametes and mothering embryos may be very different. Populations of *S. oleracea* are strongly protandrous yet because the plants are dioecious the protandry cannot be explained as an outbreeding device. If it is interpreted as one manifestation of ecological differentiation between the sexes it raises the further question of whether protandry and protogyny in hermaphrodites are really outbreeding mechanisms or whether they represent a somewhat similarly evolved non-synchrony of demand on limiting resources but occurring, in hermaphrodites, between the two gamete types within the plant or within a flower.

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Table 4 Effect of plant density on precocious death of male plants of *Spinacia oleracea*: percentage of male and female plants dead at harvest 17 August

Density	Mean (g)		s.e. of difference between the two means	Degrees of freedom
	♂	♀		
Low	13.62	0	2.03*	6
Medium	16.93	0	4.10†	6
High	22.31	0	0.9300*	6

Significance levels: *0.001; †0.01.

Table 5 Mean percentage of leaves dead at harvest (17 August) on surviving male and female plants of *Spinacia oleracea* sown at three densities

Density	Mean (g)		s.e. of difference between the two means	Degrees of freedom
	♂	♀		
Low	44.93	33.87	4.6122	6
Medium	59.45	34.46	4.8369*	6
High	53.56	28.29	2.5520†	6

Only leaves more than 2 cm long were recorded.

Significance levels: *0.01; †0.001.

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Toad tadpoles associate preferentially with siblings

Bruce Waldman & Kraig Adler

Section of Neurobiology and Behavior, Cornell University, Langmuir Laboratory, Ithaca, New York 14850

Highly structured social systems can involve close kinship groups (eusocial insects¹, lion prides², helpers-at-the-nest in birds³). However, kinship generally has not been considered to be important or necessary for what seem to be merely gregarious phenomena, such as schooling behaviour⁴. In fact, Fisher first proposed kinship theory to explain the evolution of noxious taste in certain gregarious insect larvae. He reasoned that traits which at first may not be directly beneficial to the individual, such as distastefulness or warning coloration, might be selected for if they conferred advantage to siblings in the swarm⁵. Like some insect larvae, the tadpoles of many toads (genus *Bufo*) are conspicuously coloured, distasteful to predators and highly gregarious, forming densely packed schools in open areas along pond shores⁶. The tadpoles' aggregative behaviour may be important for feeding efficiency^{7,8}, thermoregulation⁹ or predator deterrence⁸, but their conspicuousness coupled with their distastefulness suggest that schooling may also serve an aposematic function. Consistent with the predictions of a kin selection model, we report here that tadpoles of the American toad (*Bufo americanus*) preferentially associate with siblings in laboratory conditions.

During May and June 1977, we collected amplexant pairs of toads from several breeding congregations. Each pair was held in the laboratory in a separate 10-l bucket, and most females oviposited in these buckets within 24 h. Each clutch of eggs was transferred to a separate aquarium, where tadpoles were reared on boiled spinach for about 45 d, until they reached developmental stage 35 (ref. 10). Tadpoles were then placed for 24 h in 0.00025% aqueous solutions of either methylene blue or neutral red. This staining technique resulted in minimal mortality and marked the translucent fins of the tadpoles for several weeks, allowing accurate identification of sibling groups by visual inspection.

To determine whether tadpoles aggregate with siblings, for each test, 25 tadpoles from one clutch were marked blue, and 25 tadpoles from another clutch were marked red. The 50 tadpoles were then released at randomised positions and allowed to acclimatise for 24 h in an indoor test pool filled to a depth of 3 cm with water. Subsequently, for test periods ranging from 4 to 7 consecutive days, the positions of all tadpoles in the pool and their clutch-identifying colours were recorded twice each day, in the morning and afternoon; thus, each test consisted of 8–14 trials. During each trial a grid of sixteen 0.05-m² partitioned sections was lowered into the pool and the positions of all individuals were marked on an acetate sheet fastened over the grid. The partitions limited the movement of the tadpoles during the 90-s period in which data were recorded.

The coordinate positions thus obtained were analysed by comparing the distance of each tadpole to its nearest sibling neighbour with the distance to its nearest non-sibling neighbour. If they aggregate preferentially with siblings, one would expect that tadpoles should be closer to siblings than to non-siblings. This prediction was confirmed in all six experimental tests, using different groups of tadpoles in each test (Table 1). For each test, the mean nearest-neighbour distance between siblings was significantly less than the distance between non-siblings (Fig. 1). Analysis of variance showed this sibling effect to be highly significant ($P < 0.0001$, F -test), whereas variation within experimental tests, among tests and in interactions between effects was not significant (Table 2). The among-tests effect approaches significance, perhaps due to different aggregative tendencies among tadpoles used in different tests.

The results of separate analyses of individual trials further substantiate these findings (Table 1). In the majority of the experimental trials (65%; $n = 62$), the nearest-neighbour distance between siblings was significantly less than that between non-siblings (Table 1). Although discrete aggregations of sibling groups were not always apparent, in 37% of all trials the mean coordinates of the two groups differed significantly (Table 1), indicating that the sibling groups separated out into different areas of the pool. Moreover, the tadpole groups were not simply attracted to different fixed regions of the pool, as their positions changed in sequential trials (Fig. 2). This suggests that the tendency for tadpoles to be closer to siblings than to non-siblings cannot be explained by differential responses of the two groups to possible environmental gradients in the pool.

To control for the possibility that these results were caused by either the marking or testing procedures, we carried out tests in which all 50 tadpoles were from the same clutch. Half of these individuals were stained blue and half red; all other procedures were followed precisely as in the experimental tests. In the two control tests the nearest-neighbour distances between same-coloured tadpoles were not significantly less than those between

Table 1 Group identity and analysis of separate trials within each test

Test No.	Sibling groups		No. of trials per test	No. of trials where sibling distance < non-sibling distance ($P < 0.05$)	No. of trials where mean coordinates differ ($P < 0.05$)
	Red	Blue			
Experimentals					
1	B	A	8	6 (75%)	2 (25%)
2	A	C	12	7 (58%)	1 (8%)
3	A	B	14	8 (57%)	5 (36%)
4	D	A	10	8 (80%)	6 (60%)
5	E	D	10	5 (50%)	4 (40%)
6	D	E	8	6 (75%)	5 (62%)
				No. of trials where same-colour distance < different-colour distance ($P < 0.05$)	
Controls					
7	D	D	8	0 (0%)	0 (0%)
8	D	D	8	0 (0%)	1 (12%)

In the experimental tests, egg clutches were obtained from five different pairs of parents (A–E) from four separate ponds (Tompkins County, New York). Pairs A and D were from the same pond; linear distances between the ponds of origin for the other pairs of parents were: A to B, 11 km; A to C, 3.7 km; D to E, 2.6 km. In the control tests, all groups of tadpoles were obtained from the same clutch. Unlike Fig. 1, where whole tests are compared, the individual sequential trials within each test were analysed with respect to (1) whether the distance between siblings was less than that between non-siblings (Wilcoxon signed-ranks test, one-tailed¹⁹), and (2) whether the mean coordinates of the two sibling groups differed (Mann-Whitney U -test, two-tailed¹⁹). For example, test 1 consisted of eight trials, in six of which siblings were significantly closer to each other than to non-siblings. In two of the eight trials in test 1 the centres of density (=centroids) of the two groups differed. For this analysis, the centres were considered different if their positions along either the north–south or east–west axis differed significantly.

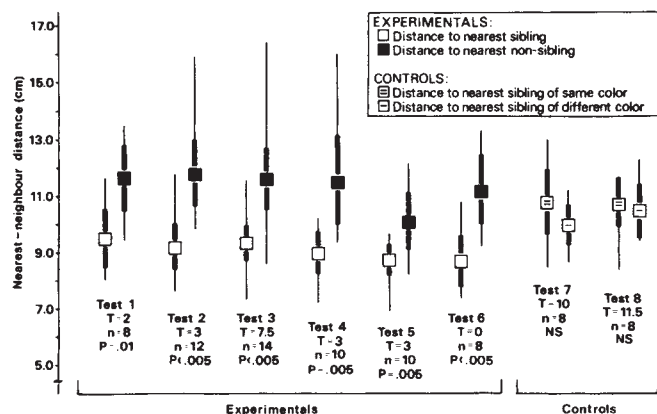


Fig. 1 Mean nearest-neighbour distance between sibling and non-sibling tadpoles (experimentals) and between siblings of the same and different colours (controls). Rectangular bars around means (symbols) represent 95% confidence intervals; vertical lines indicate ranges. Probability levels are given below the data for each test (T , n and P represent the test statistic sample size [number of pairs of trials] and level of significance, respectively). In all six experimental tests, individuals were significantly closer to siblings than to non-siblings ($P \leq 0.01$; Wilcoxon signed-ranks test). In control tests all tadpoles were siblings; same-coloured individuals were not significantly closer to each other than they were to differently coloured tadpoles ($P > 0.05$; Wilcoxon signed-ranks test).

differently coloured tadpoles (Fig. 1). Thus, the possibility that tadpoles in the experimental tests merely positioned themselves near same-coloured individuals can be discounted.

These results provide the first experimental evidence that a lower vertebrate is capable of sibling recognition. The tadpole distributions we found could arise if (1) siblings are directly attracted to one another, or (2) tadpoles avoid individuals they perceive to be non-siblings, perhaps due to aggressive encounters. We do not know what sensory modalities tadpoles use in making this discrimination, nor do we know how genetic and experiential factors effect this response. Siblings develop within a continuous jelly egg mass, and on hatching remain near the remnants of the jelly mass for several days. During these critical early developmental stages, tadpoles might 'learn' to identify siblings. Indeed, a facilitative social environment may be fundamental to the development of most normal sibling interactions¹¹. Recognition of siblings might also be modifiable by experience later in life¹². Clearly, sibling recognition need not be based solely on genetic factors.

Preferential association of tadpoles with siblings in ponds could result in the formation of sibling schools. Within schools, individuals can 'hide' from predators, thus decreasing the likelihood that they will be preyed on^{13,14}. Yet to gain this advantage, individuals need not school with siblings. Indeed, the more adaptive strategy for the individual would seem to be to sur-

round itself with non-siblings so that neither it nor its siblings are taken by a predator. However, if tadpoles associate with siblings, as our results suggest, their schooling behaviour may represent more than just 'selfish' cover-seeking. A predator that strikes a schooling individual may learn that tadpoles are distasteful, and thus it would avoid preying on other members of the school. The tadpoles' black body colour might function as a warning signal to further reinforce this 'lesson'. These traits, although not directly beneficial to the prey individual if it is eaten, may be selected for if they provide protection to related individuals in the school which are likely also to carry alleles for these altruistic traits¹⁵. The alarm pheromone released by injured *Bufo* tadpoles may represent another kin-selected trait, as the signalling individual is always wounded^{16,17}.

A sibling recognition mechanism could evolve in the following manner. In one individual, a mutation arises for distastefulness. The individual metamorphoses, survives to reproductive age, and produces young, several of which now carry the allele for distastefulness. As tadpoles associate in sibling groups for several days after hatching, because of their relative immobility, the loss of any of these distasteful individuals to a predator will benefit siblings, and thus distastefulness can spread by means of kin selection. Even if the unpalatability of a single larva is not enough to deter a predator, the cumulative noxious effect resulting from eating several larvae might be strong enough to cause future predator aversion to the school¹⁸. In a similar manner, aposematic coloration could spread. At this stage the benefits of distastefulness are high, but as the tadpoles begin actively swimming, and mixing with non-siblings becomes possible, the benefits become reduced or even negative. Next, a mutation arises for a sibling-association mechanism and is favoured because it prolongs the benefit of distastefulness and

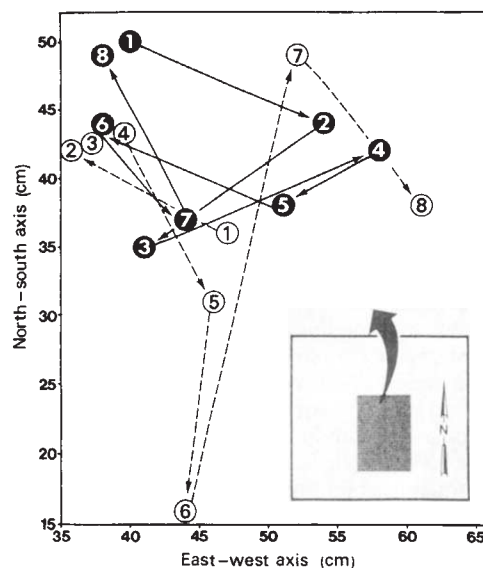


Fig. 2 Movement patterns of two sibling groups of tadpoles in a representative test. Centres of density given here are from test 6; the two sibling groups are group D (solid symbols and lines) and E (open symbols and dashed lines). The numbers inside symbols and the arrows show the sequence of trials in which the test was carried out. The two groups differed significantly in position along either the north-south or east-west axis during trials 1, 2, 3, 4 and 6 ($P < 0.05$; Mann-Whitney U -test, two-tailed). The inset shows the test pool, 88x96 cm, and the region of the pool in which the centres of density of the two groups were located (shaded area). A more detailed inspection of the sequential centres of density (trials 1-8) for the two sibling groups shows that each sibling group moved throughout the pool. Tadpoles were not fed during a test, nor were conditions in the pool altered. There was no evidence in this test or in the others that the separate groups preferred particular fixed regions of the pool.

Table 2 Analysis of variance for experimental tests

Source of variation	Degrees of freedom	Sum of squares	F Value	P
Sibling effect	1	77.39	44.06	<0.0001
Time effect (within tests)	7	14.49	1.15	0.36
Test effect (among tests)	5	21.97	2.44	0.06
Sibling x Test	5	5.29	0.59	0.71
Time x Test	35	71.54	1.13	0.36
Sibling x Time	7	6.88	0.55	0.79
Residual error	35	63.07	—	—

Three sources of variation and their interactions were considered. 'Sibling' refers to variation in nearest-neighbour distance dependent on whether or not that neighbour was a sibling. 'Time' refers to variation among sequential trials within single tests; only the first eight trials per test were used. 'Test' refers to variation among the six experimental tests.

aposematism into later developmental stages. Those individuals which discriminate siblings from non-siblings can preferentially associate with siblings, and thus can effectively direct their altruism towards siblings. This evolutionary scenario does not necessitate an error-free discrimination system; individuals which show any preferential sibling aggregative tendencies should have greater fitness than those which school indiscriminately with siblings and non-siblings. Although work is still in progress to identify the mechanisms by which sibling recognition is accomplished, our results are consistent with the predictions of a kin selection model.

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Ca²⁺ is essential cofactor for trypanocidal activity of normal human serum

Jan D'hondt, Nestor Van Meirvenne, Luc Moens & Masatoshi Kondo*

Laboratorium voor Serologie, Instituut voor Tropische Geneeskunde, B-2000 Antwerpen and
Departement Celbiologie, Universiteit Antwerpen, B-2610 Wilrijk, Belgium

Normal human serum has been known to exert a cytotoxic effect on *Trypanosoma brucei* subspecies for nearly 80 yr¹. But in spite of many attempts, no trypanocidal factor was found in human or baboon serum²⁻⁶, until Rifkin demonstrated a high density lipoprotein (HDL) in normal human serum with trypanocidal activity⁷. The conclusion that this was the trypanocidal factor was supported by the report that serum from patients with Tangier disease, characterised by a severe deficiency of HDL, lacked trypanocidal activity⁷. We report here that Ca²⁺ is an essential cofactor for the trypanocidal activity of normal human serum, in which α_2 macroglobulin (α_2) might function as a Ca²⁺-carrier. We further show that D-glucose, D-fructose and D-mannose can suppress the trypanocidal action of normal human serum, whereas glycerol has the opposite effect.

We used four clones of *T. brucei* subspecies. Clones of the variable antigen types (VATs) AnTat 1 and 8 were derived from a stock of *T. brucei brucei*⁸. A serum sensitive (S) and a serum

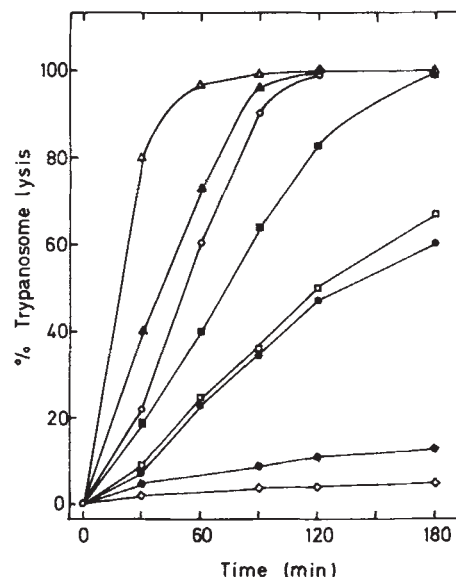


Fig. 1 Effect of dialysis, temperature and Ca²⁺ on trypanocidal activity of normal human serum. Serum was dialysed against PSG or BSG at 4 °C for 24 h with a minimal change of the volume of dialysate. The column-separated *T. b. brucei* AnTat 8 (10⁶ cells per 20 μ l) was incubated with 980 μ l serum at 36 °C for the length indicated in the figure and cell lysis was followed as described in the text. When added, 50 μ l of a stock solution of Ca²⁺ or Mg²⁺ was used in 930 μ l serum to give a final concentration of 3.2 mM. $\circ$, Undialysed serum; $\triangle$, plus Ca²⁺; $\bullet$, PSG-dialysed serum; $\blacktriangle$, plus Ca²⁺ or $\square$, plus Mg²⁺; $\blacksquare$, BSG-dialysed serum; $\diamond$, heat-treated serum (60 °C, 45 min); $\blacklozenge$, plus Ca²⁺.

resistant (R) clone of VAT ETat 2 were derived from a stock of *T. brucei rhodesiense*⁸⁻¹¹. A large number of cryo-stabilates of each clone was prepared by passage through mice (female OF1 mice) and stored under liquid nitrogen¹². Trypanosomes were collected from mice 48 h after infection, purified by passage through a DEAE-cellulose column¹³, washed and resuspended in PSG (50 mM sodium phosphate buffer, pH 8, 36.4 mM NaCl and 83 mM glucose) or BSG (48 mM sodium borate buffer, pH 8, 77 mM NaCl and 83 mM glucose). Human serum samples were collected from healthy volunteers, pooled and used immediately for assays or stored at -70 °C until use. *In vitro* assay for the trypanocidal activity of serum was performed by incubating parasites (10⁶ cells per ml) at 36 °C in PSG or BSG (unless otherwise noted) in the presence of human serum at the concentration specified in the figure legends. Percentage cell lysis was determined by counting the cell ghosts among at least 500 cells under a phase contrast microscope.

Normal human serum, after dialysis against PSG for 24 h, had considerably less trypanocidal activity on *T. b. brucei* AnTat8 than did undialysed control serum, reaching only about 60% cell lysis even after 3 h incubation (Fig. 1). However, the addition of 3.2 mM Ca²⁺ to dialysed serum resulted in a marked restoration of the trypanocidal activity (Fig. 1). The extent of restoration was clearly dependent on the concentration (1.6-12.8 mM) of Ca²⁺ added (Fig. 2). Dialysis against PSG caused a more effective reduction of serum trypanocidal activity than did that against BSG, further implying an involvement of Ca²⁺ in this cytotoxic activity (Fig. 1). Ca²⁺ stimulated the trypanocidal activity, of not only dialysed but also undialysed serum, eliminating an initial lag period at high concentrations of Ca²⁺ (Figs 1 and 2). Another divalent cation, Mg²⁺, had a negligible effect on the trypanocidal activity of PSG-dialysed normal serum (Fig. 1).

Furthermore, serum deprived of its trypanocidal activity by heat inactivation (45 min at 60 °C) could not be reactivated to a significant extent by the addition of 3.2 mM Ca²⁺ (Fig. 1). Thus the restoration of trypanocidal activity by Ca²⁺ requires the

* To whom correspondence should be addressed at Departement Celbiologie, Universiteit Antwerpen.

Table 1 Effect of carbohydrates on trypanocidal activity of normal human serum

Parasite	Control		% Cell lysis in the presence of							
	PB	HS	D-glucose		D-fructose		D-mannose		glycerol	
			PB	HS	PB	HS	PB	HS	PB	HS
<i>T. b. brucei</i> AnTat 1	100	87	1	25	2	27	0	16	0	97
<i>T. b. brucei</i> AnTat 8	100	96	0	35	0	39	0	26	2	100
<i>T. b. rhodesiense</i> ETat 2 S	100	64	0	14	2	19	0	12	0	94
<i>T. b. rhodesiense</i> ETat 2 R	100	2	0	0	0	0	0	0	1	34

Trypanosomes (10^6 cells per ml) were incubated at 36 °C for 2 h in 72 mM (final) phosphate buffer (PB, pH 8) or 50% (v/v) normal human serum (HS), containing 145 mM (final) carbohydrates as indicated in the table, except controls where 145 mM PB alone or 50% (v/v) human serum diluted with 154 mM NaCl was used.

presence of an intact trypanocidal factor in the serum, and Ca^{2+} alone cannot exert the cytotoxic effect. In addition, trypanosomes suspended in BSG containing 3.2 mM Ca^{2+} were not affected after 3 h of incubation at 36 °C (not shown).

Human $\alpha_2\text{M}$ is a protein ligand for Ca^{2+} (and also for other metal ions) affecting various physiological and immunological reactions¹⁴. Thus it was conceivable that $\alpha_2\text{M}$ in human serum might function at least in part, in addition to free serum Ca^{2+} , as a Ca^{2+} -carrier for a trypanocidal factor. Moreover, a fraction containing trypanocidal factor, reported by Yamaguchi³ and Rifkin¹⁵, contained both HDL and $\alpha_2\text{M}$. To test this possibility, normal human serum was first treated with rabbit anti- $\alpha_2\text{M}$ antiserum, removing all immunologically detectable $\alpha_2\text{M}$ but leaving normal amounts of HDL and low density lipoprotein (LDL), and then dialysed against BSG for 24 h. As Fig. 2 shows, the dialysed serum had no trypanocidal activity on *T. b. brucei* AnTat 1, while the addition of 0.8 mM Ca^{2+} restored 60–70% of the activity detected in complete serum. This suggests the active involvement of $\alpha_2\text{M}$ in the trypanocidal reaction, possibly as an effective Ca^{2+} -carrier, whose bound Ca^{2+} cannot easily be removed by either dialysis or the addition of EGTA or EDTA (not shown) to human serum. Rifkin's conclusion¹⁵ that no difference in trypanocidal activity was found between serum

absorbed with either rabbit anti- $\alpha_2\text{M}$ IgG or normal rabbit IgG can be explained by the fact that no dialysis was performed to eliminate free serum Ca^{2+} after treatment with anti $\alpha_2\text{M}$ IgG and sufficient Ca^{2+} (about 2 mM) was present in the *in vitro* assay system. Indeed a maximal trypanocidal activity coincided with the Sepharose 6B column fractions of LDL-free serum, where HDL and $\alpha_2\text{M}$ overlapped each other¹⁵.

The bloodstream form of *T. brucei* species can utilise D-glucose, D-fructose, D-mannose and glycerol efficiently as carbon and energy sources but depends exclusively on exogenous carbohydrate because it can not accumulate intracellularly storage polysaccharides¹⁶. We examined the effect of these carbohydrates on serum trypanocidal activity towards all four *T. brucei* clones, and the results are summarised in Table 1. First, they protected the parasite suspended in 145 mM sodium phosphate buffer (pH 8) from lysis during 2 h of incubation at 36 °C. Trypanosomes suspended in buffer without an energy supply were lysed within 10–20 min. Second, the presence of either D-glucose, D-fructose or D-mannose markedly suppressed the trypanocidal activity of normal serum in a concentration dependent manner (not shown). Interestingly, glycerol enhanced trypanocidal activity to some extent, even with the resistant clone of *T. b. rhodesiense* ETat 2 R (Table 1). Thus a simple energy supply to trypanosomes by carbohydrate metabolism is not responsible for altering the capability of trypanocidal factor.

Gruenberg *et al.*¹⁶ have demonstrated, by kinetic analysis of transport systems, that D-glucose and glycerol do not share the same carrier site in the bloodstream form of trypanosomes of *T. brucei*. The first event for trypanolysis by human HDL should be the binding of HDL to the membrane of trypanosome, which may be mediated by a specific surface receptor, as has been shown for serum LDL¹⁸. Thus it is tempting to suggest that human HDL utilises the specific carbohydrate carrier sites for its interaction with the trypanosome membrane. The carrier site for D-glucose, while involved in its transport, cannot interact efficiently with a trypanocidal factor, whereas the carrier site for glycerol enhances such an interaction. Ca^{2+} requirement for the trypanocidal activity might be mediated, among other possibilities, by its binding to the phospholipid moiety of HDL. The subsequent event involves the alteration of the membrane structure, possibly by removing lipid components as postulated by others⁷. Because subspecies of *T. brucei*, *T. b. rhodesiense* and *T. b. gambiense*, and *T. b. brucei*, cause sleeping sickness in humans and nagana in cattle, respectively, the elucidation of the mechanism by which human HDL causes trypanolysis could contribute to the development of effective prevention and treatment of trypanosomiasis.

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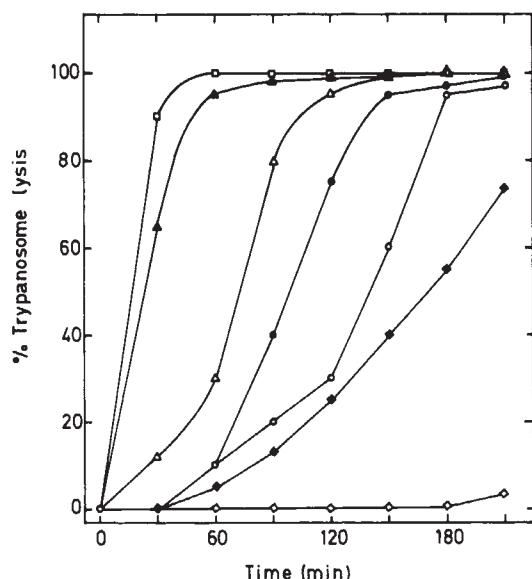


Fig. 2 Effect of Ca^{2+} and $\alpha_2\text{M}$ on trypanocidal activity. Serum was treated with rabbit anti-human $\alpha_2\text{M}$ antiserum (1.5 mg IgG per ml antiserum, Cappel Laboratories, Cochranville). Both untreated and treated ($\alpha_2\text{M}$ -deficient) sera were dialysed against BSG at 4 °C for 48 h. The purified *T. b. brucei* AnTat 1 (10^6 cells per 20 μl) was incubated at 36 °C with 980 μl 50% (v/v) serum or serum containing various concentrations of Ca^{2+} as indicated in the figure. Cell lysis was followed as in Fig. 1. $\circ$, BSG-dialysed untreated serum; $\bullet$, plus 1.6 mM Ca^{2+} ; Δ , plus 3.2 mM Ca^{2+} ; $\blacklozenge$, plus 6.4 mM Ca^{2+} or $\square$, 12.8 mM Ca^{2+} ; $\diamond$, BSG-dialysed treated serum; $\blacklozenge$, plus 0.8 mM Ca^{2+} .

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Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line

David P. Aden*, Alice Fogel*†, Stanley Plotkin‡, Ivan Damjanov§ & Barbara B. Knowles*

* Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

‡ Wistar Institute of Anatomy and Biology, Department of Pediatrics, Children's Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104

§ Department of Pathology, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

A significant aspect of primary hepatic carcinoma in man is the high positive correlation of hepatocellular carcinoma with infection with hepatitis B virus (HBV)¹. Analysis of the relationship between HBV infection and oncogenesis is difficult because natural infection with HBV is limited to man and experimental infection has been achieved only in chimpanzees and gibbons. Furthermore, because HBV has not been successfully propagated in cell culture, basic study of virus-cell interaction of the aetiological agent of one of the most widespread infections of man has been impossible². Recently, however, a cell line (PLC/PRF/5) derived from a human hepatoma biopsy was described³ which produces the HBV surface antigen (HBsAg) and so provides a tool for the experimental investigation of HBV *in vitro*. We now report the derivation and characterisation of two additional cell lines from primary liver carcinomas. In contrast to the PLC/PRF/5 cell line, these cell lines retain the capacity to synthesise many human plasma proteins, including both albumin and α -fetoprotein (AFP). One of these lines also produces HBsAg. We also present evidence that HBsAg synthesis and secretion in this cell line are correlated with the growth state of the culture. This finding is in contrast to the continuous HBsAg production found in the PLC/PRF/5 cell line.

The cell lines were established from liver tumour biopsies obtained during extended lobectomies of a 15-yr-old caucasian male from Argentina (1975) and an 8-yr-old black male from the US (1976). Tumour minces were cultured on irradiated mouse cell layers (STO). The cell lines established after these biopsies had been cultured for several months were designated Hep G2 and Hep 3B, respectively, and their morphological characteristics and epithelial cell shape are compatible with those of liver parenchymal cells. Histology of the liver biopsies revealed well differentiated hepatocellular carcinoma with a trabecular pattern. At autopsy of the second patient a portion of the lung, containing suspected metastases, was cultured. The only cells which grew from these tissue fragments were of fibroblastic morphology and are designated Hep 3L.

Supernatants from Hep 3B, Hep G2, PLC/PRF/5, Hep 3L and STO cell lines were tested for the presence of human

albumin and AFP by Ouchterlony two-dimensional gel diffusion using commercially obtained antisera. Only the supernatants from the Hep G2 and Hep 3B lines formed precipitin lines which gave a reaction of identity with purified human albumin and human serum and purified human AFP and human placental cord serum, respectively. Human albumin and AFP were analysed quantitatively by radioimmunoassay inhibition testing (Fig. 1). Samples from supernatants of these same cultures were also tested for production of HBsAg, those from Hep 3B were positive whereas supernatants from Hep G2, Hep 3L and STO cultures were repeatedly negative. PLC/PRF/5 supernatants served as a positive control.

Ultrastructural examination of cells from the Hep 3B line was performed using standard electron microscopic techniques. The cells resembled previously described human and experimental liver cell carcinomas⁴. The cells contained prominent cisterns of granular endoplasmic reticulum and a moderate amount of mitochondria; stacks of annulate lamellae and aggregates of glycogen were found in the cytoplasm. No viral particles or cytoplasmic alterations indicative of HBsAg were noted in either apparently healthy or necrotising cells or in the cellular debris.

While monitoring the Hep 3B cultures during the first 1.5 yr of its growth in culture, we observed that the quantity of HBsAg in the supernatants was variable, ranging from undetectable to large amounts. In contrast, supernatants from the PLC/PRF/5 were always positive for HBsAg even within 24 h of subculturing. The same Hep 3B cultures that were variably positive for HBsAg were consistently positive for human albumin. To determine the cause of this variation we monitored the kinetics of HBsAg, AFP and albumin production in a series of replicate

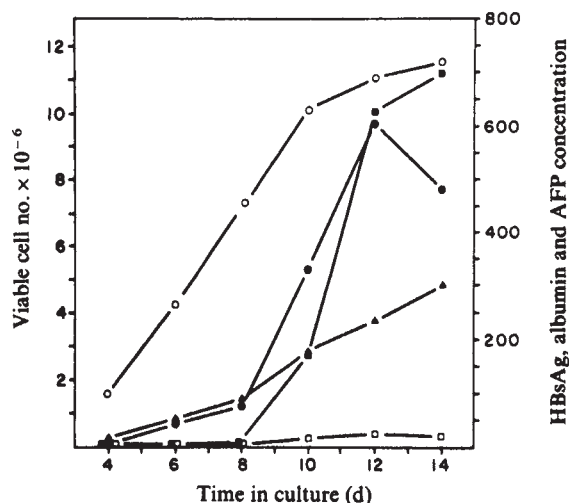


Fig. 1 T75 flasks containing confluent monolayers of Hep 3B cells were tested for HBsAg; those containing >300 ng of HBsAg were used to establish eight replicate cultures. T75 flasks containing monolayers of irradiated feeder layer (STO) cells were seeded with 10^6 viable hepatoma cells in single cell suspension (0.25% trypsin, 1 mM versene, then washed once with medium) and fed with 15 ml Eagle's minimal essential medium (AutoPow, Flow) containing 10% fetal bovine serum (Armor, heated to 56°C for 0.5 h) and 10 mM glutamine. At days 4, 6, 8, 10, 12, 14, the supernatant fluid was removed, centrifuged and frozen, single cell suspensions prepared as above and viable cells determined by the erythrosin-B dye exclusion method. The remaining cells were washed three times in Dulbecco's modified phosphate-buffered saline, centrifuged, and frozen and thawed four times in 1 ml distilled H₂O. These total cell lysates were also frozen until assayed. Control for each assay included supernatant fluid and/or cell lysates from cultures of Hep G2, Hep 3L, PLC/PRF/5 and STO. HBsAg was quantitated using the AUSRIA II (Abbott) solid phase radioimmunoassay (RIA) kit and comparing positive values to a standard curve with purified HBsAg, determined as part of the same assay (test sensitivity of 0.65 ng ml⁻¹). Albumin and AFP were quantitatively determined in competitive inhibition RIAs by comparing values to standard curves obtained in the same tests with known concentrations of human albumin (Pentex) and AFP (test sensitivity of >20 ng ml⁻¹). Supernatants and cell lysates from STO and Hep 3L were negative for HBsAg, albumin and AFP, Hep G2 samples were negative for HBsAg, and PLC/PRF/5 samples were negative for albumin and AFP. ■, ng HBsAg in supernatant; □, in cell lysate; ▲, μg albumin; ●, μg AFP; ○, viable cell number.

† Present address: Central Virus Laboratory, Ministry of Health, Tel-Aviv, Israel.

cell cultures by collecting supernatants, determining cell number and preparing cell lysates at various time points. As shown in Fig. 1, HBsAg is not detectable in either supernatants or cell lysates during the first 6 days of subculture, but can be detected by 8 days (11 ng per culture). During this interval, the cell number has increased consistently. HBsAg increased rapidly from day 8 to day 12, whereas the cell number less than doubles during this same time interval. In contrast, albumin is detectable at all time points, increasing with cell number and time in culture. AFP can be found in supernatants throughout the culture growth period, although the increase in the rate of AFP production paralleled that of HBsAg. Cell lysates quantitatively analysed for these proteins gave no evidence for a large internal pool of any of the proteins (Fig. 1, data not shown for albumin and AFP). HBsAg was found in detectable concentrations in cell lysates at the same time as in the supernatants, showing that its rapid increase in the supernatant was not due merely to release of previously synthesised protein.

Culture conditions were examined in an attempt to account for the delay in HBsAg production. To determine whether cell contact is necessary for HBsAg production, the same number of cells were plated throughout a tissue culture dish or restricted to a small portion of the dish by seeding into a plastic ring which was removed 24 h later. No effect of immediate cell contact on the kinetics of HBsAg production was found (Table 1); HBsAg was detected on the same day post-plating and the cultures containing cells in close contact did not produce more HBsAg.

Second, we examined the possibility that a component of fresh cell culture medium hindered HBsAg production or that a cellular product secreted into the medium was necessary for HBsAg production. Feeding of cultures with conditioned medium obtained either from pre-HBsAg positive cultures of Hep 3B (Table 2) or from other human and mouse cell lines (data not shown) did not result in early initiation or increased production of HBsAg, although conditioned medium was capable of supporting cell multiplication (see Table 2 legend for details of culture conditions). When the culture medium is replaced daily with fresh medium, the production of HBsAg is delayed and the quantity reduced (Table 2).

There are several possible mechanisms to explain the delayed appearance of HBsAg in the Hep 3B cell line. The simplest is that the antigen is continuously produced by each cell but that the assay system precludes detection until large amounts of HBsAg have accumulated. This is unlikely because of the sharp rise in the concentration of HBsAg as the cells approach the stationary phase of the growth cycle (Fig. 1). The lowest concentration of HBsAg antigen detectable in this system is approximately 1 ng per culture. As Hep 3B cultures containing 10^7 cells are capable of producing an average of 220 ng of HBsAg within a 24-h period, HBsAg would be detected if it was being continuously synthesised by each cell in the early cultures.

A second possibility is cell cycle control as observed in plasma protein biosynthesis by fetal rat hepatocytes⁵. These studies clearly demonstrated that the synthesis of albumin is independent of the growth state of the cells; the rate of production and release is constant throughout the growth cycle. AFP production by rat hepatocytes, on the other hand, is a function of the

Table 2 Effect of fresh and conditioned medium on HBsAg production by Hep 3B

Culture conditions	Days after subculturing				
	4	6	8	10	15
Conditioned medium*	0§	9	9	5 (0.4)	NT
Fresh medium†	0	6	27	121 (3.6)	169
Fresh medium changed daily‡	0	0	1	10 (0.4)	26

1.5×10^6 cells per 75 cm² per 12 ml medium subcultured in replicate flasks in the conditions listed below. Samples of supernatant fluid were recovered from the cultures on indicated days following subculture and frozen at -20°C until assay for HBsAg. Duplicate cultures allowed computation of ng HBsAg produced per 10^6 cells on day 10. NT, Not tested; conditioned medium, although capable of supporting cell growth ($>11 \times 10^6$ cells on day 10), could not support these cells for 15 days.

* Conditioned medium was obtained from 5-day-old cultures of Hep 3B (1.5×10^6 cells plated on day 0; 5×10^6 cells collected on day 5) which were not yet HBsAg positive; this was used to subculture Hep 3B cells. 6 ml were removed every 2 days and replaced with 5-day conditioned medium.

† 6 ml were removed every 2 days and replaced with fresh medium.

‡ Total supernatant fluid removed every day and replaced with fresh medium.

§ ng HBsAg per culture.

^{||} ng HBsAg per 10^6 cells.

position of the cultures in the cell cycle. The curve (Fig. 1) for production of albumin in the human hepatoma-derived line Hep 3B reflects the results observed with rat hepatocytes; albumin is produced and accumulates in the culture supernatant fluid and a plateau is reached when the nutrient state of the culture declines. However, the kinetics of production of AFP and HBsAg by the Hep 3B cells suggests cell cycle control with production occurring when the cells stop dividing and enter the G_0 or resting phase of the cell cycle. This would explain both the appearance of HBsAg in the late log phase of culture growth (Fig. 1) and why cells repeatedly stimulated with fresh medium produce only small quantities of HBsAg. A third possible explanation is that cells in the culture differentiate or mature into terminal cells that are HBsAg producers. Experiments designed to distinguish between the last two possibilities are in progress. Interestingly, in the PLC/PRF/5 cell line, HBsAg is produced throughout the growth cycle (ref. 3 and data not shown).

Speculation on the control of HBsAg synthesis in cell lines must take into consideration the possibility that the viral sequences may be carried as episomal transcriptional units or integrated into the genome of the host. The PLC/PRF/5 genome is host-cell integrated⁶ and preliminary evidence indicates that this is also the case for the Hep 3B cell line. As the site of integration may regulate the transcription of the viral information, the controlled production of HBsAg might be indicative of different integration sites in the Hep 3B and PLC/PRF/5 cell lines. Investigation of cell lines derived from human hepatomas, including those negative for HBV expression, should continue to yield insight into the relationship between HBV and human hepatocellular carcinoma.

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Table 1 Effect of cell contact on HBsAg production by Hep 3B

Culture conditions	Days after subculturing			
	6	8	10	15
Unrestricted	0*	0	4	40 (26) [†]
Restricted	0	0	2	28 (18.4)

0.3×10^6 cells were plated in 19-cm² culture dishes containing 4 ml of culture medium. Unrestricted cultures allowed growth throughout the plates; restricted cultures were established by placing the Hep 3B cells in a 3-cm² plastic ring which was removed 24 h later.

* ng HBsAg per culture.

† ng HBsAg per 10^6 cells.

Hepatitis B surface antigen produced by a human hepatoma cell line

Jacinta Skelly, Jennifer A. Copeland, C. R. Howard & A. J. Zuckerman

Department of Medical Microbiology, and WHO Collaborating Centre for Reference and Research on Viral Hepatitis, London School of Hygiene and Tropical Medicine, London WC1, UK

Infection with hepatitis B virus is associated with the appearance in the serum of a specific antigen, hepatitis B surface antigen (HBsAg), a serologically complex structure present on the coat of the double-shelled 42-nm hepatitis B virus (HBV) particle. HBsAg is also present, often in large excess, in the form of small, spherical 20–25-nm particles in the sera of infected individuals¹. The serologically distinct core (HBcAg) or nucleocapsid of the 42-nm virus is 27 nm in diameter and contains double-stranded circular DNA with a molecular weight (MW) of $1.6\text{--}2.0 \times 10^6$, and an associated DNA polymerase². A third antigen, hepatitis B e antigen (HBeAg), appears to correlate with the number of virus particles³ and the relative degree of infectivity of surface antigen-positive sera⁴, is frequently detected free in the serum and has been shown to be closely associated with the viral core⁵. Failure to propagate hepatitis B virus serially in tissue culture is a major obstacle to precise biochemical characterisation of the antigens and the development of vaccines against this important infection. The use of purified HBsAg 20–25-nm particles prepared from the sera of persistently infected donors for the immunoprophylaxis of hepatitis B is being investigated⁶. Such preparations have been shown to protect susceptible chimpanzees on challenge with HBV^{7,8}. There are, however, problems associated with the collection of HBsAg positive sera from persistently infected carriers. The recent establishment of a HBsAg-producing cell line from a human hepatoma^{9,10} has provided an alternative source of HBsAg particles with the added advantage that both the production and quality of the antigen may be more precisely controlled. The availability of this cell line also facilitates the study of HBV gene products by biochemical methods applicable to cultured cells, in the absence of contaminating serum proteins. In this study, the 20–25-nm HBsAg particles produced by these cells were characterised and compared with those derived from the sera of persistently infected human carriers.

A study in our laboratory of the rate of HBsAg production showed that it was minimal during logarithmic cell growth, and maximal towards the end of a 7-day maintenance period (J.A.C. *et al.* in preparation). Titres of up to $1.3 \mu\text{g ml}^{-1}$ of HBsAg were obtained by incubating confluent cultures in maintenance medium for 7 days. Measurements of HBsAg, HBeAg and DNA polymerase by the most sensitive methods currently available¹¹ were performed on cell culture fluids, and on pellets derived by ultracentrifugation of the fluids (Table 1). Only HBsAg was detectable. Electron microscopy revealed that the predominant form was the 20–25-nm spherical particle. Pelleted HBsAg was further purified by two cycles of isopycnic banding in CsCl as described previously¹². The buoyant density determined during this procedure was 1.19 g ml^{-1} , as found by others¹³. Material banding at this density was radiolabelled with the Bolton and Hunter reagent to a specific activity of $5 \mu\text{Ci } \mu\text{g}^{-1}$ before further purification¹⁴. It was then subjected to centrifugation in linear gradients of Urografin¹² (Fig. 1). The majority of the radiolabel was not present in the surface antigen, as only about 10% of it was present in the region of the gradient (fractions 10–14) in which serum surface antigen was found in parallel gradients. Furthermore, when the fractions were tested

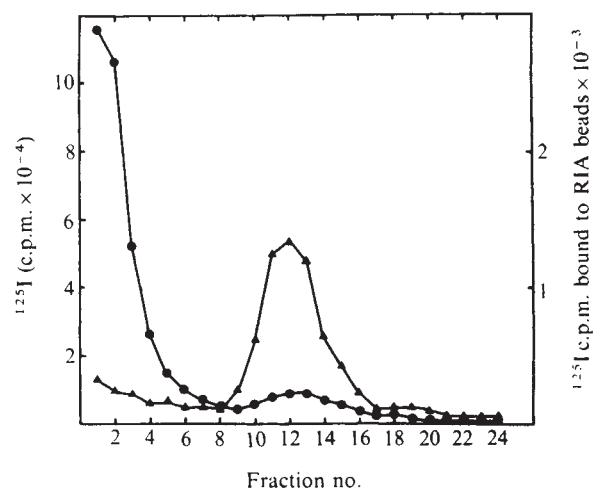


Fig. 1 Centrifugation of radioiodinated, partially purified HBsAg. Antigen was layered on to discontinuous 10–25% gradients of Urografin buffered with 0.01 Tris HCl, pH 7.3, and centrifuged at 52,000g for 18 h at 4°C in a Beckman SW 27.1 rotor. Fractions (0.7 ml) were collected and 10- μl aliquots of each were counted in an LKB Ultragamma counter. Fractions containing radiolabelled HBsAg were detected by direct adsorption to solid phase anti-HBsAg. Aliquots (10 μl) of each fraction were mixed with 200 μl 10% normal human serum in phosphate-buffered saline (PBS) and incubated with antibody-coated radioimmunoassay beads (AUSRIA II, Abbott) for 3 h at 40°C. The beads were then rinsed with 10 ml of water and counted. ●, Total c.p.m.; ▲, c.p.m. bound to anti-HBsAg. Centrifugation is from left to right.

for radiolabelled HBsAg by adsorption to beads coated with hepatitis B surface antibody (anti-HBs), only fractions 10–14 were positive. These fractions were combined but attempts made to separate the antigen from Urografin by gel filtration¹² or sedimentation were unsuccessful.

In contrast to serum HBsAg, there was evidence of breakdown of the hepatoma antigen particles subjected to these treatments, as shown by failure to recover most of the radioactivity in the void volume or pellets. An alternative to rate-zonal centrifugation was therefore adopted. An affinity adsorbent column was prepared consisting of rabbit anti-HBs IgG adsorbed to protein A-Sepharose. Control experiments showed that HBsAg did not bind to protein A-Sepharose alone, or in the presence of non-immune rabbit serum. Material radiolabelled after the second CsCl step was applied to the column. After extensive washing to remove unbound radiolabel, the IgG and bound antigen were eluted with 3 M sodium thiocyanate (NaSCN). The polyacrylamide gel profiles of the radiolabelled material before affinity chromatography, and of the fraction bound by anti-HBs are shown in Fig. 2a and b, respectively. The four major polypeptides of the material before affinity chromatography had MWs of 73,000, 64,000, 54,000 and 31,500. In contrast, the MWs of the polypeptides of the affinity-purified antigen were 47,000, 34,000, 28,000 and 23,000. The latter two polypeptides co-migrated with major polypeptides of serum HBsAg run in a parallel gel. The antibody affinity step therefore allowed the recovery of the small fraction of ^{125}I -labelled material with a density of 1.19 ml^{-1} which is HBsAg reactive and uncontaminated by the other constituents of this material.

The results confirm that the HBsAg produced by the PLC/PRF/5 line is very similar in size, morphology and buoyant density to the 20–25-nm spherical particles of circulating serum HBsAg. The purification procedure developed for serum HBsAg¹² was readily adaptable to the purification of hepatoma

HBsAg from tissue culture fluids. In spite of the fact that tissue culture supernatant contains considerably less protein than serum, our results showed that the hepatoma HBsAg required extensive purification. After two equilibrium bandings in CsCl, at least 90% of the ^{125}I -labelled protein was not HBsAg. The bulk was material which remained at the top of a Urografin rate-zonal gradient (Fig. 1) and consisted of polypeptides with MWs of 73,000, 64,000, 54,000 and 31,000 (Fig. 2). Hence, it was necessary to devise a suitable final purification step, as recovery of the antigen from the Urografin was low; also the purity and specificity of the final product before polypeptide analysis needed confirmation. Both of these aims were attained by binding of labelled HBsAg to anti-HBs IgG adsorbed to a protein A-Sepharose column. It is concluded that the final product is HBsAg for several reasons: first, it is specifically adsorbed by anti-HBs IgG; second, it contains no trace of the major polypeptides of the contaminating material; and third, it contains two polypeptides of the serum surface antigen¹⁵⁻¹⁷. Note that the hepatoma surface antigen does not contain the higher MW polypeptides frequently detected in serum HBsAg: in particular there is no polypeptide in the MW range of serum albumin which has been identified as a constituent of highly purified preparations of serum HBsAg. This observation is in agreement with our finding that the hepatoma HBsAg does not bind to Blue Sepharose CL-6B and with the observation of R. Dodd (personal communication) that the hepatoma cells produce little or no albumin. In particular, the finding of polypeptides with MWs of 28,000 and 23,000 in hepatoma HBsAg reinforces the association of these two polypeptides with HBsAg

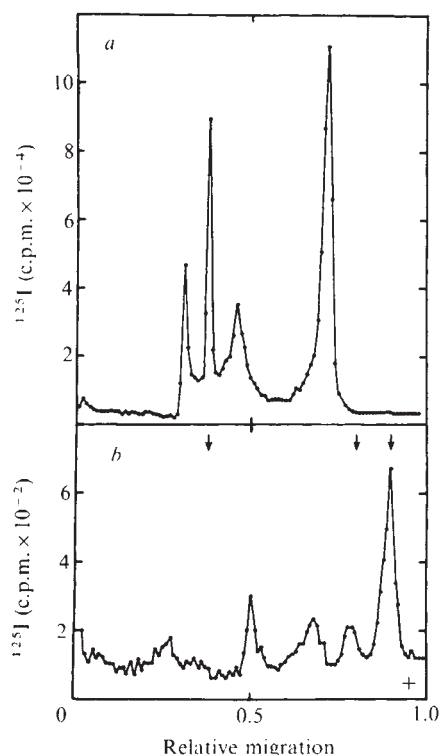


Fig. 2 SDS polyacrylamide gel electrophoresis of material radioiodinated after two isopycnic centrifugations in CsCl (a) and of the 3 M NaSCN eluate from the protein A-Sepharose-anti-HBsAg column (b). The conditions for disruption and electrophoresis in 10% cylindrical gels were as previously described¹² except that the stock acrylamide solution contained 0.8% w/v methylene bisacrylamide. Radioactivity was determined by counting 1-mm slices. The arrows indicate the positions of p64, p28 and p23 of serum HBsAg run in a parallel gel^{12,15}.

Table 1 Hepatitis B antigens and DNA polymerase in tissue culture fluid from the PLC/PRF/5 line

	Culture fluid	Pellet (75 × concentrate)
HBsAg	581	6,350
HBeAg	1.25	1.22
HBcAg	1.07	0.95
HBcAg after disruption	1.13	1.21
DNA polymerase	0.65	0.91

Measurements of HBsAg, HBcAg, HBeAg and DNA polymerase PLC/PRF/5 culture fluids, and in pellets derived by ultracentrifugation. Fluids were collected from confluent monolayers of cells after 7 days of incubation in maintenance medium (minimal essential medium, containing 1% fetal calf serum.) After a preliminary clarification by low-speed centrifugation, the fluids were centrifuged at 83,000g for 18 h in a Beckman SW27 rotor and resuspended in 1/75th the original volume. HBsAg was detected using a commercial radioimmunoassay system (AUSRIA II, Abbott) and HBeAg by a similar procedure using polystyrene beads coated with human IgG positive for anti-HBe. The same reagent was used for the preparation of the radioligand. HBcAg was measured similarly using human sera positive for anti-HBc. For this latter test, a duplicate sample was also treated with 1% NP40 and 0.1% 2-mercaptoethanol to detect the presence of HBcAg within 42-nm virus particles. DNA polymerase was measured as previously described¹⁹.

* c.p.m. in sample/c.p.m. in control.

activity, which was previously demonstrated by Triton X-100 fractionation of serum material¹⁵. The other polypeptides sometimes found associated with serum HBsAg^{17,18} as opposed to hepatoma HBsAg may be tightly bound serum components such as albumin¹⁵, or components of HBsAg produced by normal rather than malignant liver cells. It is not possible to decide between these two alternatives at present.

The simple biochemical composition, and ready availability of HBsAg from the PLC/PRF/5 cell line make this line attractive as a possible source of material for intact particle or subunit vaccines for hepatitis B.

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Tumour promoter alone induces neoplastic transformation of fibroblasts from humans genetically predisposed to cancer

Levy Kopelovich, Nancy E. Bias & Lawrence Helson

Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021

Neoplastic transformation is a multi-phase process apparently caused by carcinogens and subject to the influence of promoters¹⁻⁴. The naturally occurring phorbol esters⁴⁻⁶ such as 12-O-tetradecanoyl phorbol-13-acetate (TPA) are potent tumour promoting agents. Through the use of phorbol esters a two-stage process of malignant transformation has been demonstrated in the mouse skin model⁴⁻⁷ and, more recently, in cell culture systems⁸⁻¹⁰. Studies *in vitro* suggest that TPA reversibly inhibits terminal differentiation in most¹⁰, but not all model systems, and that its function is presumably to increase the probability of expression of the malignant phenotype¹⁰. We have studied the effects of TPA on mutant human fibroblast cell strains derived from individuals with hereditary adenomatosis of the colon and rectum (ACR), an autosomal dominant trait¹¹. We have previously demonstrated in these fibroblasts abnormal phenotypic expressions which often appear in transformed cells¹²⁻¹⁷. In these studies, we have assumed that the ACR cell exists in an 'initiated state' due to a dominant mutation and that expression of the malignant state might only require treatment with a promoting agent^{18,19}. This simple experimental protocol provided a novel system for the study of cancer promotion *in vitro*^{20,21}. We have now demonstrated, for the first time, the growth *in vivo* of human mutant cells exposed to TPA alone.

Subepidermoid biopsy specimens were taken from flat skin of normal appearance from ACR individuals and normal volunteers at the Memorial Sloan-Kettering Cancer Center¹². Skin fibroblasts (SF) that grew out around the explant as a monolayer were isolated and carried in culture. The cells were grown in Earle's minimum essential medium and Earle's balanced salt solution (GIBCO) supplemented with 2 mM glutamine, 1% non-essential amino acids, penicillin (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and 10 mM HEPES buffer, pH 7.25 (EMEM) in the presence of 15% fetal calf serum (FCS). The cell strains were used between the 5th and 12th passage and were routinely checked for bacteria and mycoplasma.

The protocol for obtaining the cells used in the nude mouse inoculation experiments is shown in Fig. 1. Cells were plated in 75-cm² flasks at an initial density of 4×10^5 cm⁻², and TPA (100 ng ml⁻¹) in 0.01% dimethyl sulphoxide (DMSO) was added at day 1 post-plating. Near-confluent cultures of these TPA-treated cells were continuously passaged in culture at a subculture density of $4-6 \times 10^3$ cell cm⁻² (about 10-14 d per passage). On each passage, SF from a parallel subculture were plated in agar in the presence of 100 ng ml⁻¹ of TPA and examined for colonies 4 weeks later. SF from another culture were injected bilaterally into the anterior chamber of the eye of athymic mice at a concentration of about 1×10^6 cells per 0.02 ml phosphate-buffered saline (PBS) per eye.

Table 1 describes the following cell cultures injected into the athymic mice: a DMSO-treated ACR cell culture (ME), a TPA-treated ACR cell culture (ME) which did not grow in agar during seven consecutive passages in liquid culture (Fig. 1), and a TPA-treated ACR cell culture (PF) which grew in agar (Ag I) and has been isolated from agar-growing clones on passages 2 and 4 for further studies (Fig. 1)^{20,21}. As shown in Table 1, the two TPA-treated cell cultures gave rise to tumours in athymic mice, but only the PF cells were descendants of TPA-induced

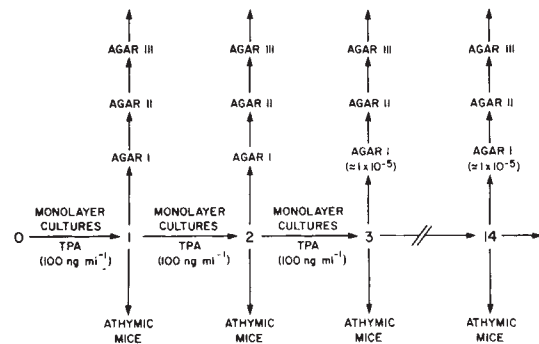


Fig. 1 The protocol for TPA-treated cells. Agar plates were prepared essentially as described elsewhere³⁰. Nutrient agar (Difco), 1.84% (w/v), was melted in boiling water and equilibrated at 45 °C. It was then mixed with $2 \times$ EMEM-30% FCS containing 200 ng ml⁻¹ TPA/0.02% DMSO 1:1 (v/v) at 45 °C, and 4 ml were plated on to each of 3-5 60-mm dishes as an underlayer and allowed to firm for 15 min at room temperature. Another portion of this solution was mixed with a cell suspension of 5×10^4 cells ml⁻¹ in 1 $\times$ EMEM 1:1 (v/v) containing 100 ng ml⁻¹ of TPA in 0.01% of DMSO or DMSO alone. This preparation (4 ml), representing 1×10^5 cells per dish, was plated over the base layer and allowed to firm for 1 h at room temperature in a chamber irrigated with 5% CO₂. Agar colonies were scored microscopically after about 4 weeks of incubation at 37 °C in a humidified 5% CO₂ incubator. Inoculation of the nude mice (CD-1) was carried out with cells that were trypsinised, counted and washed twice with PBS to give a final cell concentration of 1×10^6 per 0.02 ml of PBS. A volume of 0.02 ml was injected under a magnifying glass bilaterally into the anterior chamber of the eye. The mice were checked every week for the appearance of tumours. These usually became apparent at 2 weeks post-injection and were removed 1-2 weeks later. Each number represents a consecutive passage.

anchorage-independent colonies (Fig. 1; Ag I). To date, we have not obtained tumours with DMSO-treated ACR cells, DMSO-treated normal cells or TPA-treated normal cells (Table 1).

At about 4 weeks post-inoculation, the optic nerve was severed and the intact eye was removed and placed in 10% formaldehyde for histological examination. A cross-section through the eye (perpendicular to the optic nerve-cornea axis) shows the occurrence of an apparently well differentiated tumour, characterised by uniformly appearing, highly basophilic, fibroblast-like cells (Fig. 2). Tumours were obtained in all mice injected with the TPA-treated ACR cells, although not always bilaterally (Table 1).

We have previously demonstrated an unusual biphasic dose-response relationship for cultured human SF to TPA^{20,21}, perhaps suggesting that TPA affects at least two distinct processes of cell proliferation—one which is inhibitory to cell growth, but can be saturated at relatively low concentrations, and another at the higher concentration range, which stimulates cell proliferation. Whether these results suggest the existence of at least two cell populations each of which displays a distinct type of receptor for TPA, or a single cell population with at least two types of receptor for TPA remains to be established. Consequently, in the present report we chose to study the malignant transformation of ACR cells at a high TPA concentration (100 ng ml⁻¹).

The chronic application of TPA also effects a loss of anchorage sensitivity of ACR cell cultures^{20,21}. Cells selected for anchorage independence might presumably respond primarily to the growth-stimulating ability of TPA and this fraction may then conceivably be enriched by serial transfers in agar²¹. However, we found that growth in agar due to TPA occurs at a low frequency, cannot be serially sustained beyond two passages in agar (Fig. 1, Ag II) and is variable for a given human cell strain during consecutive passages in liquid culture^{20,21}. For example, the human strain PF has given rise to anchorage-independent colonies at passages 2 and 4, but not at passages 1, 3, 5 and 6.

Table 1 Effect of TPA on the tumorigenicity of ACR cells

Cell strains and treatment	Dose-response pattern to TPA*	Growth in agar	Tumours formed
DMSO/HC†	NA	—	0/4
TPA/HC†	Bimodal	—	0/3
DMSO/ME‡	NA	—	0/3
TPA/ME‡	Bimodal	—	4/4 (4)
TPA/PF§	Bimodal	+	3/3 (2)

TPA was always applied at a concentration of 100 ng ml⁻¹ in 0.01% DMSO and was changed twice weekly. Controls received 0.01% of DMSO alone. TPA-treated subconfluent cultures were split at a density of 4–6 × 10³ cells cm⁻². Colonies retrieved from agar were expanded in liquid cultures in the presence of TPA until enough cells had become available for inoculation in the nude mice. NA, not applicable.

* See refs 20, 21, and text.

† Represent SF from normal individuals. The dose-response curve of normal SF to TPA is apparently bimodal, but these cells are about 50–100-fold more sensitive to TPA than are ACR cells²⁰. The latter may be due to a significant reduction in receptor sites for TPA and/or a change in the affinity of binding of TPA to its receptors in ACR cells.

‡ Represent SF from individuals with the Gardner variant of familial polyposis coli (ACR).

§ Represent SF from a clinically asymptomatic individual who is one of the F₁ progeny of an ACR pedigree and who is positive by our tests^{12–22} for this trait. To date, no differences have been observed in our studies between the general case of ACR and the Gardner variant.

|| Figures in parentheses indicate the number of mice with tumours occurring in both eyes.

This same cell strain did not grow in agar in three subsequent experiments. The human strain ME, which in this study did not grow in agar at all during seven consecutive passages, has been previously shown to grow in agar at a similar frequency to PF. However, both cell strains formed tumours all the time. The results suggest a high degree of heterogeneity in the human cells which is more apparent during selection of the TPA-induced anchorage-independent phenotype than during the process of tumour formation in athymic mice. In other words, the probability of a TPA-induced tumour is considerably higher than that of a TPA-induced anchorage independence in our system (Table 1). The results may further suggest that a loss of anchorage dependence is not necessarily a prerequisite of tumour growth and that these two processes may be modulated in parallel by separate control mechanisms²². In this regard, a TPA-induced permanent loss of anchorage dependence has been demonstrated with stable 'initiated' mouse epidermal cells in culture²³.

TPA has been shown to enhance the stable transformation of murine, and more recently, of human foreskin fibroblasts previously exposed to a carcinogen^{8,9,24,25}. Thus, our results may indicate that the ACR mutation is a complete one for malignancy, representing an initiated state²², and that the chronic application of TPA, in support of the two-stage 'Berenblum-hypotheses' (ref. 4) can precipitate the final oncogenic event. Alternatively, this cell mass growing *in vivo* may represent an intermediary state, similar perhaps to the TPA-induced stable papillomas in the mouse skin model^{4–7} or to the clinical appearance of polyps in the colon. The latter would suggest that an additional mutation(s) is necessary for the malignant transformation of ACR cells with certain promoters acting during all phases of oncogenesis to increase the probability of expression of the malignant phenotype.

The possibility that chromosomal restructuring is associated with promotion of malignancy by TPA^{26–28} in our cell system remains to be established. In this respect, an elegant analysis of lymphoma development that proposes diversity of initiation followed by convergent cytogenetic evolution has recently been enunciated²⁹. The way in which the presence of TPA in ACR cells might effect a transition from the initiated state to the malignant state is now being investigated.

We thank Dr Robert A. Good for suggesting the eye as an immune-privileged site in the nude mouse system, Dr I. Bernard

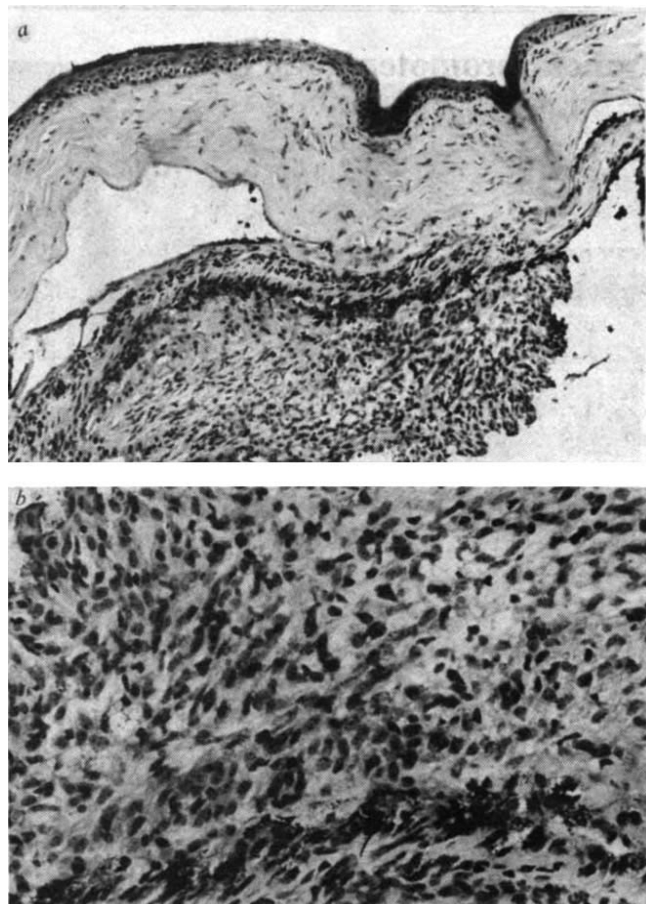


Fig. 2 A histological section of a tumour through the anterior chamber of the eye. *a*, Eyes were severed at the optic nerve and placed *in toto* in a 10% formaldehyde solution for 48 h. Cross-sections were made perpendicular to the cornea-optic nerve axis. ×90. *b*, As in *a*, magnified ×225. The pigmented particles (lower part) arose from the iris during sectioning in the periphery of the eye.

Weinstein for helpful discussions, Mrs C. Helson for help in the inoculation of the nude mice, and Ms P. Monaghan and Ms R. Vuolo for technical assistance. This work was supported in part by grant CA 19259 from the National Large Bowel Cancer Project, and NCI grant CA 08748 and contract N01 CP 43366. *Note added in proof:* We have recently demonstrated that TPA-treated ACR cells which grew in athymic mice showed about 33% increase in cell aneuploidy (L.K. and R. Moon, unpublished). These chromosomal aberrations, if stable, may conceivably be consistent with a somatic mutation(s).

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Galactosyltransferase and membrane glycoprotein abnormality in human platelets from Tn-syndrome donors

J. P. Cartron

U76 INSERM, Centre National de Transfusion Sanguine,
6, rue Alexandre Cabanel, 75739 Paris Cedex 15

A. T. Nurden

U150 INSERM, Hôpital Lariboisière, 2, rue Ambroise Paré,
75475 Paris Cedex 10, France

Early studies on the analysis of membranes isolated from the erythrocytes of Tn-patients by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed a severe reduction in the staining capacity of glycophorin with the periodate-Schiff (PAS) reaction^{1,2}. A low sialic acid and galactose (Gal) content of the polyagglutinable red cells was confirmed³ while it was reported^{2,4} that the abnormal red cells of Tn-patients contained little or no UDPGal: GalNAc- β -3-D-galactosyltransferase (T-transferase) activity. The glycoprotein (GP) abnormality in Tn-erythrocytes appeared to be due to incomplete synthesis of the alkali-labile oligosaccharide chains of glycophorin². We now report studies on the membrane GP composition and the T-transferase activity of platelets isolated from three Tn-syndrome patients whose red cell membranes contain GP abnormalities which are typical of those found in this rare clinical condition^{1,2}.

There is increasing evidence that the unmodified presence of certain membrane glycoproteins is vital for the normal haemostatic functioning of blood platelets⁵. The disaccharide β -D-galactosyl (1-3)*N*-acetyl-D-galactosamine, which is the determinant of the T-antigen, has been demonstrated on the surface of human red cells^{6,7} and platelets⁸. In normal human erythrocytes the T-antigen is substituted with sialic acid. The pathological exposure of the T-antigen or α -glycosidically linked *N*-acetyl-D-galactosamine (GalNAc) residues may lead to the polyagglutination of the red cells, as antibodies against the exposed antigens are present in most adult sera⁹. The polyagglutination resulting from the exposure of α -GalNAc residues forms the basis of the Tn-syndrome¹⁰. The frequent association between the exposure of Tn-receptors on red cell membranes and a low platelet count¹⁰ suggested that similar modifications might also be present in the platelets of Tn-donors.

Platelets from each patient were washed and analysed by SDS-PAGE. The GP profiles of platelets isolated from patient Ba are compared with those of normal human platelets in Fig. 1. Major changes were observed in the GP pattern of the Tn platelets. The individual PAS-staining platelet glycoproteins contain different amounts of inter- and/or intramolecular disulphide bonds and exhibit characteristic changes in migration on SDS-PAGE that are dependent on the state of reduction of the disulphides¹¹. Analysis of the non-reduced GP profiles clearly showed that the GP Ib/Is band was primarily affected in the Tn platelets, with its PAS-staining capacity being markedly

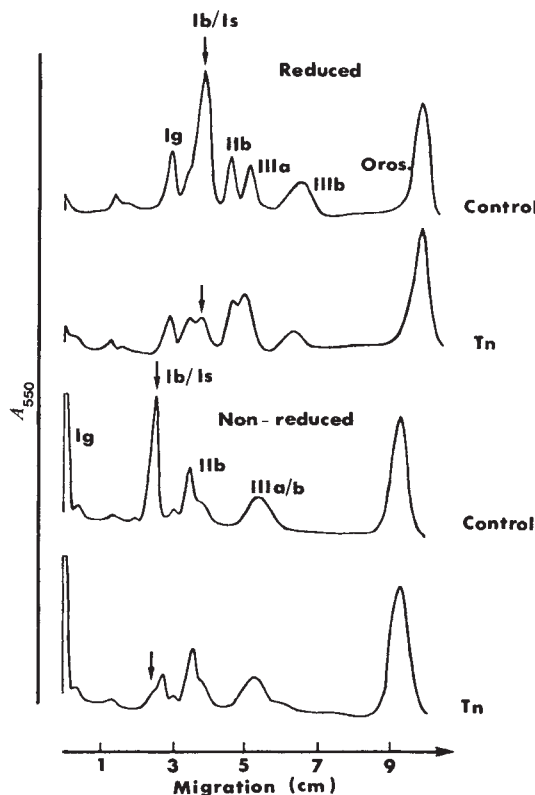


Fig. 1 A comparison of the glycoprotein profiles of normal human and Tn platelets. Washed platelets were isolated as previously described¹⁷ and resuspended at $2 \times 10^9 \text{ ml}^{-1}$ in 0.01 M Tris-HCl, pH 7.0, containing 0.15 M NaCl, 3 mM EDTA and 5 mM *N*-ethyl maleimide. The platelets were solubilised by heating at 100 °C for 5 min following the addition of one fifth volume 12% SDS. SDS-PAGE was performed either following reduction of disulphide bonds at 37 °C for 1 h, in the presence of 5% v/v 2-mercaptoethanol (reduced samples, upper profiles) or in the absence of disulphide bond reduction (non-reduced samples, lower profiles). Electrophoresis was performed according to the procedure of Laemmli²⁰ using 10.5 $\times$ 0.5 cm polyacrylamide separating gels containing 6% acrylamide 0.2% bis (reduced samples) or 7% acrylamide 0.2% bis (non-reduced samples). Protein (400 μg) was applied to each gel together with 5 μg orosomucoid. Glycoproteins were located by the PAS reaction¹⁷ and the gels scanned densitometrically at 550 nm. The orosomucoid acted as an internal standard for the PAS reaction. Glycoprotein profiles of platelets isolated from Tn donor Ba are illustrated. Arrows indicate the position of the Ib/Is peak.

decreased. The residual GP Ib/Is also migrated slightly faster on SDS-PAGE, suggesting that it was of a lower than normal molecular weight. The criteria used for the identification of the PAS-bands labelled in Fig. 1 are detailed elsewhere^{12,13}. GP Ib, IIb, IIIa and IIIb are membrane glycoproteins. Following SDS-PAGE of unfractionated normal platelets the PAS-staining intensity of the GP Ib band is increased relative to that of the other membrane glycoproteins^{12,13}. GP Is refers to the fraction of this band that has been shown to be released from the surface of washed platelets during homogenisation; it has also been termed glycocalicin¹⁴. GP Ib and Is may represent two fractions of a structurally similar glycoprotein¹⁴. GP Ig refers to an intracellular, granule-localised, glycoprotein¹².

The SDS-PAGE glycoprotein profile of the platelets of a second Tn-syndrome patient, Az, is shown in Fig. 2. In this profile the residual GP Ib/Is peak was slightly greater than was observed with the platelets of the donor Ba. We² have provided evidence for the presence of a dual population of erythrocytes in the blood of most Tn individuals, that is to say a subpopulation of erythrocytes with normal GP profiles and normal T-transferase activity coexisted with the pathological red cells. To

investigate the possible presence of a dual population of platelets in Tn donors and that the abnormal GP profiles of Tn platelets was accompanied by the exposure of GalNAc residues on the platelet surface, aliquots of platelet rich plasma from patients Az and Du were separated on affinity columns of Sepharose 6MB-*Helix pomatia* lectin. This lectin specifically recognises terminal GalNAc groupings¹⁵. Typical SDS-PAGE glycoprotein profiles of the platelet subpopulations which either bound (HP+ve) or were not retained by the column (HP-ve) are shown in Fig. 2. The HP-ve subpopulation gave a normal GP profile, while analysis of the HP+ve platelets revealed little PAS-staining in the GP Ib/Is position.

Initial studies demonstrated the presence of T-transferase activity in lysates prepared from the platelets of normal donors (Table 1), the level of activity being independent of the ABO blood group. T-transferase activity was strongly reduced in lysates prepared from washed platelets isolated from each of the three Tn-donors studied. When the total population of platelets isolated from donor Du (with 30% of the normal level of platelet T-transferase activity) were separated into the HP-ve and HP+ve subpopulations, practically all of the T-transferase activity was located in the HP-ve platelets. As illustrated in Fig. 2 for the donor Az the HP-ve platelets had a normal GP composition, whereas little PAS-staining was observed in the GP Ib/Is position for the HP+ve platelets. Incubation of Tn-platelet lysates for 18 h at 37 °C with purified T-hapten resulted in no detectable Gal release and no evidence of an increased galactosidase activity.

Glycoprotein abnormalities have previously been reported in two congenital platelet disorders, Glanzmann's thrombasthenia and the Bernard-Soulier syndrome^{5,12}. Little or no PAS-staining is observed in the GP IIb and IIIa positions following SDS-PAGE of thrombasthenic platelets, and recent results suggest severe molecular deficiencies of these membrane glycoproteins^{12,16}. In the Bernard-Soulier syndrome, little or no PAS-staining is observed in the GP Ib/Is position^{12,17}. As shown in Fig. 2, the HP+ve Tn platelets presented a GP profile similar to that observed with Bernard-Soulier platelets. In view of this, T-transferase activity was measured in lysates obtained from platelets isolated from a thrombasthenic and a Bernard-Soulier patient, respectively (Table 1). T-transferase activity was within the normal range in both lysates suggesting that the mechanism by which the GP abnormalities occur in thrombasthenic and Bernard-Soulier platelets is different to that found in the Tn-syndrome.

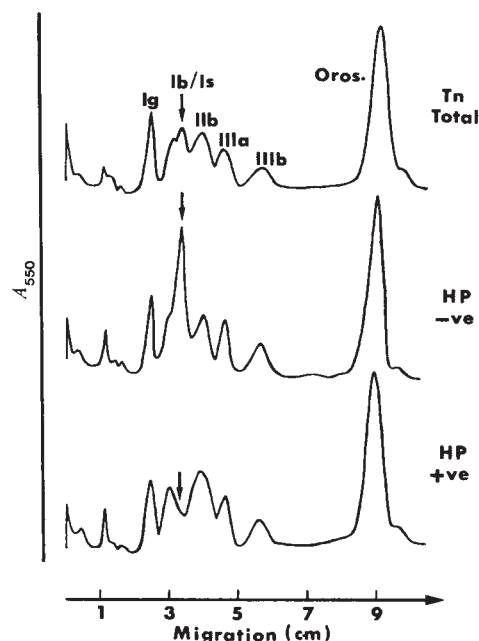


Fig. 2 Glycoprotein profiles of Tn platelet subpopulations. Platelet-rich plasma was applied to a 15 × 0.5 cm column of Sepharose 6MB-*Helix pomatia* lectin (Pharmacia) equilibrated with 0.01 M sodium phosphate, pH 7.2, containing 0.15 M NaCl, 50 µg ml⁻¹ apyrase (Sigma) and 0.5% bovine serum albumin. Platelets not retained on the column (HP-ve) were eluted directly with the above buffer, the platelets retained on the column (HP+ve) with buffer containing 0.1 M GalNAc. The isolated subpopulations and a sample of the total platelet population were washed, solubilised with SDS, incubated with 2-mercaptoethanol and their glycoprotein composition analysed by SDS-PAGE as described for Fig. 1. Glycoprotein profiles of platelets isolated from Tn donor Az are illustrated. Arrows indicate the position of the Ib/Is peak.

The low T-transferase activity in Tn-platelets strongly suggests that the GP abnormality, as in Tn erythrocytes, arises from an incomplete synthesis of the oligosaccharide chains of the glycoprotein(s) constituting the GP Ib/Is band. This conclusion is supported by the recent detection of the T-antigen in isolated human platelet membranes⁸ and the observation that binding sites for *Arachis hypogaea* lectin (which has a specificity for the T-antigen) are specifically located in the GP I position following SDS-PAGE¹⁸. To our knowledge, our results represent the first direct demonstration of a membrane GP abnormality common to both platelets and erythrocytes. They also point to possible similarities within the structure of platelet GP Ib/Is and glycophorin, and support the hypothesis that a somatic defect occurring in the primitive stem cells of haematopoietic tissue may be the underlying cause of the platelet and red cell abnormalities in the Tn-syndrome¹⁹. In common with previous reports¹⁰ each of the Tn-donors studied possessed a circulating platelet count lower than the normal level (Table 1). Whether there is any association between the platelet GP defect and the low platelet count remains to be proved. However, the specificity of the glycoprotein defect suggests that Tn platelets provide an additional model for the investigation of the possible roles of the membrane glycoproteins in platelet function.

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Table 1 UDPGal: GalNAc-β-3-D-galactosyltransferase (T-transferase) activity in human platelets

Donors	Blood group	Platelet count (10 ⁹ l ⁻¹)	T-transferase activity* (pmol mg ⁻¹ h ⁻¹)
Normal (n = 7)	A, B or O	200-400†	178 ± 28‡
Thrombasthenia Bl.C	O	200	175
Bernard-Soulier Viv.	A	70	154
Ba	O	70	13
Az	O	120	46
Tn Du whole platelets	O	53	58
Du HP-ve platelets			125
Du HP+ve platelets			17

* Measurement of the T-transferase activity was performed on platelet lysates obtained by incubating 10⁹ washed platelets in 100 µl 10 mM tris-maleate buffer pH 7.4, containing 1% v/v Triton X-100. The assay was performed in a total volume of 100 µl containing 80 µg platelet protein, 10 µmol sodium cacodylate buffer pH 6.5, 1.5 µmol MnCl₂, 0.5 µmol p-nitrophenyl-2-acetamido-2-deoxy-α-D-galactopyranoside (GalNAc-α-Nph) and 0.68 nmol UDPGal ¹⁴C-galactose (specific activity 280 mCi mmol⁻¹). After incubation for 3 h at 37 °C, the reaction product ¹⁴C-Gal-β(1-3)GalNAc-α-Nph (T-hapten) was isolated by paper chromatography and quantitated by liquid scintillation counting as previously described².

† Normal range.

‡ Mean ± standard deviation.

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Insulin binds to brain blood vessels *in vivo*

Mark van Houten & Barry I. Posner

Department of Medicine, McGill University, Montreal, Quebec, Canada H3A 2B2

The brain has generally been considered an insulin-independent organ, because insulin does not apparently exert a direct effect on brain glucose consumption¹. Recently, however, insulin receptors have been detected throughout the central nervous system (CNS) of several species^{2,3}. Since important insights into the functional significance of brain insulin receptors might be provided by identification of the cell type(s) possessing these receptors, we have attempted to localise them morphologically using light and electron microscope autoradiography. We report here results indicating that blood vessels throughout the CNS of the rat bind plasma insulin rapidly and with considerable specificity.

¹²⁵I-labelled porcine insulin (21.9 × 10⁷ c.p.m., specific activity 134 μCi μg⁻¹) in the absence or co-injected presence of graduated amounts of unlabelled insulin, or structurally dissimilar polypeptides, ovine prolactin or bovine adrenocorticotrophic hormone (ACTH), was injected intracardially into anaesthetised male rats, as previously described⁴. Five minutes after injection brains were perfused through the heart with Ringer's lactate, to wash out blood and unbound radioactivity, followed immediately by Bouin's fixative solution. Coronal 4-μm thick sections of paraffin-embedded slabs of fixed brain were stained with modified Harris' haematoxylin and eosin, and processed autoradiographically for light microscope visualisation of bound radioactivity⁵.

Light microscope autoradiography showed that, in addition to prominent reactions over the circumventricular organs, which have been described elsewhere⁴, ¹²⁵I-insulin was bound to blood vessels throughout the CNS (Fig. 1). Autoradiographic reactions occurred over brain blood vessels of all dimensions, except the large muscular blood vessels located along the perimeter of the brain.

Although the intensity of individual blood vessel reactions did not superficially appear to correlate with the size or shape of the vessel profile, there were obvious differences in the overall regional intensity of vessel reactions among different brain regions. To achieve some quantitative appreciation of these differences, the intensity of blood vessel reactions from several regions was estimated with reference to a uniform population of vessel profiles. Thus, for grain quantitation we considered only small round nucleated vascular profiles within a narrow range of

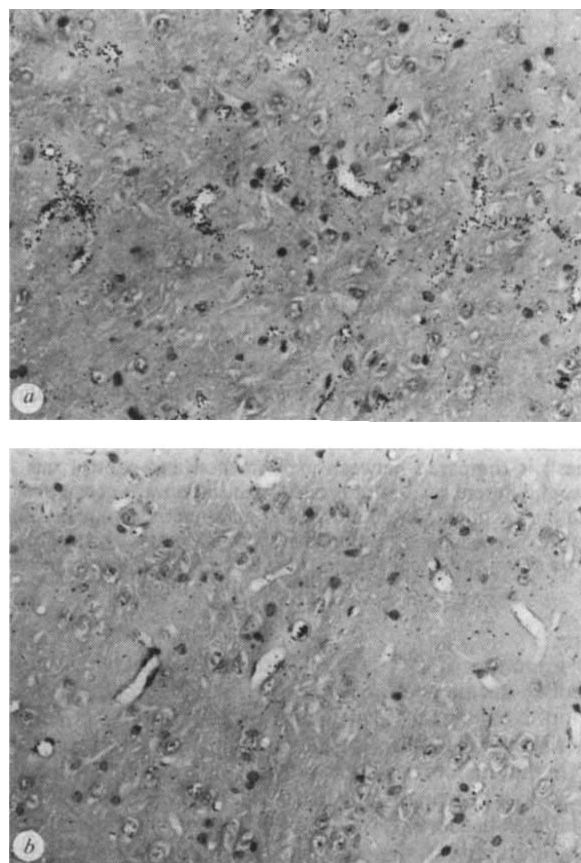


Fig. 1 The intensity of autoradiographic reactions over all neocortical blood vessels from rats injected with ¹²⁵I-insulin (a) is drastically reduced in rats co-injected with a 500-fold excess of unlabelled insulin (b). ×212; 2 month exposure.

luminal diameters (13–17 μm). These microvessels were selected to provide an index of the overall intensity of blood vessel reactions for the purpose of inter-regional comparison, as they represented units of luminal surface area which were numerous and morphologically convenient to identify under the light microscope with the aid of an ocular micrometer. The total number of silver grains over 100 of these 'standardised' microvessel profiles, scored without bias, was tallied for each of several regions of brain from rats in each injection group. The average of these totals was used to estimate variations in ¹²⁵I-insulin binding to brain blood vessels.

In rats injected with ¹²⁵I-insulin alone there was marked regional variation in insulin binding to brain microvessels (Fig. 2). The most intense autoradiographic reactions occurred in neocortex, hippocampus and hypothalamus. Less binding occurred with microvessels in cerebellum and pons, and the least binding with microvessels from white matter structures, such as corpus callosum and optic chiasm. Fortuitous inclusion of the trigeminal ganglia in our autoradiographic series revealed that microvessels in this structure of the peripheral nervous system also bind plasma insulin (Fig. 2).

Quantitation of microvessel reactions was also useful in estimating the effect of unlabelled insulin and structurally unrelated polypeptides on ¹²⁵I-insulin binding to cerebral blood vessels. Co-injection of a 500-fold excess of unlabelled insulin reduced ¹²⁵I-insulin binding to brain microvessels in all regions to about the same level (Figs 1, 2). The average reduction in autoradiographic reaction was 60–70% over grey matter microvessels, but only 40–50% over white matter microvessels, because these blood vessels bound less ¹²⁵I-insulin when this was injected alone.

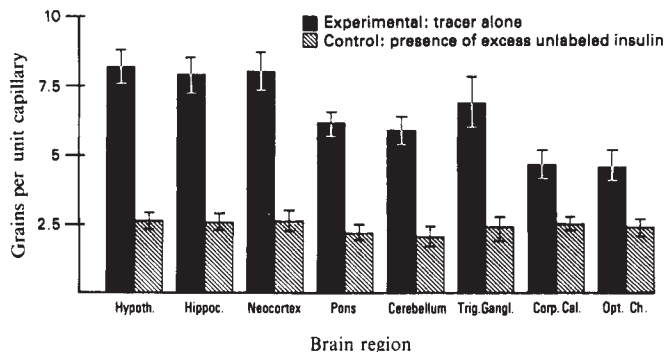


Fig. 2 Regional variations in the specific binding of ^{125}I -insulin to brain microvessels *in vivo*. The average number of silver grains ($\pm$ s.e.m.) over 100 standardised microvessels (termed 'unit capillaries') is compared between experimental and control rats for several different regions of brain. Quantitation was accomplished with the aid of an 11×11 mm ocular micrometer disk and a Zeiss Photoscope at a magnification of $\times 1600$. Estimates of background fog over non-radioactive brains showed 0.35 ± 0.15 grains per unit capillary.

Co-injection of ^{125}I -insulin with intermediate amounts of unlabelled insulin produced a stepwise reduction in the average reaction over neocortical microvessels, but co-injection of prolactin or ACTH had no effect (Fig. 3). These observations indicate that neocortical microvessels bind plasma insulin with a considerable degree of specificity. The specificity of the binding of insulin to cerebral blood vessels is confirmed by our recent autoradiographic studies showing that blood-borne ^{125}I -human growth hormone⁶ and ^{125}I -angiotensin II (ref. 7) do not bind to cerebral vasculature.

The possible cellular location of ^{125}I -insulin binding sites in association with neocortical blood vessels was tentatively identified by means of electron microscope autoradiography of the neocortex of rats injected intracardially with ^{125}I -insulin, and killed 5 min later by perfusion-fixation as before, except that a solution of mixed aldehydes served as fixing agent. Most silver grains examined in thin section autoradiographs⁸ of neocortex were situated as shown in Fig. 4 in relation to endothelium. As extensive quantitative methods^{9,10} are required to determine the precise location of the bound radiolabel, our electron microscope observations must be taken as preliminary. The present data do, however, suggest that the endothelial cell may be the site of the binding interaction (M.v.H. and B.I.P., in preparation).

The results of this study demonstrate the presence of insulin-specific binding sites in association with rat brain blood vessels.

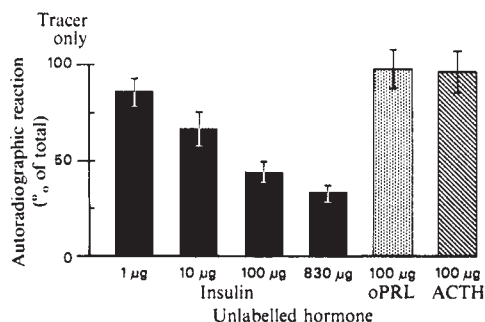


Fig. 3 Effect of unlabelled insulin and other polypeptide hormones on ^{125}I -insulin binding to neocortical microvessels. The average number of silver grains over 100 microvessels from rats injected with ^{125}I -insulin along with graduated amounts of unlabelled insulin, ovine prolactin (oPRL) or bovine ACTH is expressed as a percentage of the average number of silver grains per microvessel obtained with injection of ^{125}I -insulin alone (% of total).

All regions of brain examined showed vascular binding of insulin, although there was considerable regional variation in insulin binding capacity: the greatest insulin binding occurring with grey matter microvessels of the forebrain, and the least binding with white matter structures. It is unlikely that regional variations in insulin binding to blood vessels can be attributed to possible intrinsic differences in regional cerebral blood flow, and thus to differences in vascular accessibility to blood-borne insulin, because regional variations in autoradiographic reactions ceased when ^{125}I -insulin was co-injected with unlabelled insulin (Fig. 2). On the other hand, it is more likely that regional differences in blood vessel-associated reactions reflect regional differences in the capacity of cerebral blood vessels to bind specifically plasma insulin. This being the case, brain blood vessel binding sites for insulin may account in part for the widespread distribution of insulin receptors in the rat CNS³.



Fig. 4 Representative micrograph showing the typical location of silver grains in electron microscope autoradiographs of neocortex from a rat injected systemically with ^{125}I -insulin. The visual impression gained from examining many such grains strongly suggest that the endothelial cell is directly binding insulin. $\times 33,000$; 4 month exposure.

Indeed, the rank order of brain regions according to microvessel binding of ^{125}I -insulin *in vivo* using autoradiographic methods (Fig. 2) is strikingly similar to the rank order of regions according to receptor content, as determined by classical direct binding studies³.

Are brain blood vessels targets for the direct action of insulin? The specificity of insulin binding to brain blood vessels argues for these vessels having the recognition capacity concomitant with an ability to respond to plasma insulin. However, it is generally assumed that insulin has no direct effect of any kind on brain activity because it does not alter the rates of glucose transport across the blood-brain barrier¹. The intriguing studies of Arief *et al.*¹¹, on the other hand, suggest that insulin may directly stimulate potassium uptake by the brain *in vivo*, and that a direct action of insulin on brain electrolyte balance exerted at the level of the endothelial cell may contribute to the onset of insulin-induced seizures. Although by no means supporting this concept, the present localisation of insulin binding sites to cerebral blood vessels does raise the possibility that insulin may exert a generalised effect on brain activity by a direct action on aspects of blood-brain barrier function perhaps unrelated to glucose uptake.

In view of the possible relevance of the present observations to the general vascular complications of diabetes, it is important to determine whether insulin-receptor blood vessels exist outside the CNS. Bar *et al.*¹² recently identified genuine insulin receptors on endothelium isolated from human umbilical vein.

Autoradiographic studies similar to the ones presented here should be carried out to determine whether vascular beds in other tissues possess insulin binding sites.

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Localisation of phencyclidine-induced changes in brain energy metabolism

Richard C. Meibach, Stanley D. Glick, Russel Cox & Saul Maayani

Department of Pharmacology, Mount Sinai School of Medicine, New York, New York 10029

The abuse of phencyclidine [1(1-phenylcyclohexyl)piperidine, PCP], commonly referred to as angel dust or hog, is rapidly reaching epidemic proportions. PCP users often appear violent and increases in PCP-implicated homicides and suicides have been reported¹. In animal studies PCP has been demonstrated in brain up to 48 h after administration, long after blood levels become undetectable². However, there is little further information on the distribution of PCP within the central nervous system with regard to the possible sites of action. Recently, Sokoloff and associates^{3,4} described a new technique which can be used to visualise possible sites of drug action. The technique is based on the premise that neuronal activity is closely related to energy metabolism. Therefore, by directly monitoring 2-deoxy-D-glucose consumption before and after a pharmacological stimulus, we can obtain autoradiographic evidence of changes in neuronal activity in discrete areas of brain as a response to that stimulus. Using this procedure, we now report that PCP causes dramatic changes in glucose metabolism in very specific regions of the rat brain.

Female Sprague-Dawley rats (250 g) were used in all experiments. In pilot studies the peak dose/response of PCP as tested behaviourally in a rotometer was 5 mg per kg 40 min after intraperitoneal administration (S.D.G. *et al.*, in preparation). Thus, 40 min after PCP injection, animals were injected with 25 μ Ci of ¹⁴C-2-deoxy-D-glucose (specific activity 53 mCi mmol⁻¹, NEN), administered through a cannula implanted in the right jugular vein 24 h before injection⁵. The conscious, freely moving animals were killed 30 min later by decapitation and processed for autoradiography³.

The autoradiograms presented a striking effect of PCP on local cerebral glucose metabolism. Both increases and decreases were markedly apparent as determined by comparing the densities of the X-ray autoradiograms. The major sites of increased metabolism were components of the limbic lobe. Specifically, glucose consumption was increased in the molecular layer of the regio superior of the hippocampus. Both dorsal and ventral levels of the hippocampus were affected, although the greatest increases seemed to occur at posterior levels. This line of increased activity continued dorsally through the subicular cortex and into the retrosplenial cortex (posterior cingulate gyrus) (Fig. 1d). The anterior cingulate gyrus (Fig. 1e) and anteroventral nucleus of the thalamus demonstrated similar increases. Smaller increases in glucose utilisation were seen in a few non-limbic structures such as substantia nigra. An unusual finding was that the inferior colliculus, which normally has the highest energy demands of all brain areas³, showed a marked loss of metabolic activity (Fig. 1f).

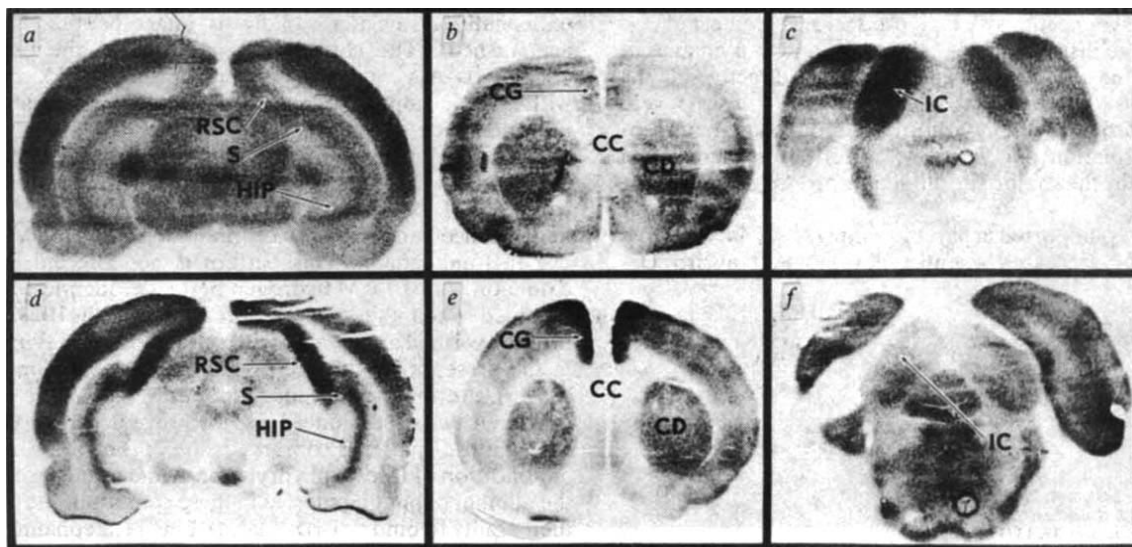


Fig. 1 Autoradiograms taken from three different levels in untreated (a-c) and PCP-treated (d-f) animals. Note line of increased intensity (glucose metabolism) in hippocampus (HIP), subiculum (S) and retrosplenial (posterior cingulate) cortex (RSC) which continues rostrally (e) into anterior cingulate cortex (CG). At the level of the midbrain (c, f) there is a dramatic decrease in intensity in the inferior colliculus (IC). CC, corpus callosum; CD, caudoputamen.

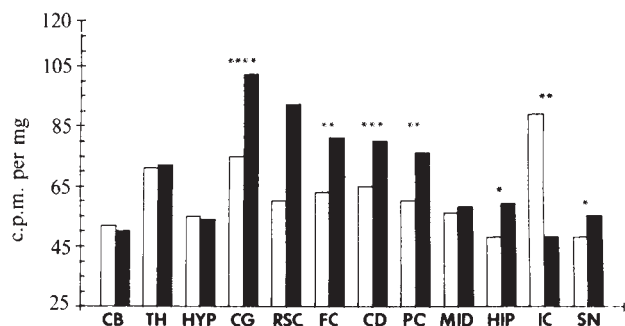


Fig. 2 A comparison of ³H-deoxyglucose uptake in untreated ($n = 7$) animals (open bars) and in animals receiving 5 mg per kg PCP ($n = 7$) 40 min before injection of ³H-deoxyglucose (solid bars). CB, cerebellum; TH, thalamus; HYP, hypothalamus; CG, anterior cingulate gyrus; FC, frontal cortex; CD, caudateputamen; PC, posterior cortex; MID, midbrain; IC, inferior colliculus; SN, substantia nigra. MID includes IC and SN, but was further dissected into IC and SN in three additional animals. The RSC was removed from only two animals. *Significantly different from normal in paired t -test, $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.005$.

In an attempt to quantify the changes in energy metabolism without using a densitometer, we repeated our experiments substituting a tritium-labelled isotope for the ¹⁴C (50- μ l injections with specific activity 40 Ci mmol⁻¹). After death, the brains were removed, weighed, dissected into 12 regions and homogenised in 1 ml sodium phosphate buffer, pH 7.4. 100 μ l aliquots (in quadruplicate) were removed, dissolved in 10 ml of NEN scintillation cocktail and 1 ml distilled water, and counted in a Beckman LS9000 scintillation counter (30% efficiency). The results of these experiments completely supported the autoradiographic data (Fig. 2); that is, the cingulate gyrus reflected the largest increase in glucose consumption (37%) whereas the inferior colliculus had the largest decrease (54%). In addition, increases in glucose consumption which were not apparent on visual inspection were found in both the frontal cortex and caudateputamen.

The present study clearly demonstrates a marked effect of PCP on the limbic system. Papez originally proposed an emotional circuit linking the cingulate gyrus, hippocampus and anterior thalamus⁶. Later studies have included the subicular cortex in these connections^{7,8}. PCP produces its greatest effects on energy metabolism in all these structures and thus enables us to visualise the functional Papez circuit directly in the autoradiograms. The fact that in humans PCP causes severe emotional disorders including disturbances of thought, perception, mood, isolation and hostility¹ is further evidence that within the brain the major site of action of PCP is within the limbic system.

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Role of disulphide and sulphhydryl groups in clustering of enkephalin receptors in neuroblastoma cells

Eli Hazum, Kwen-Jen Chang & Pedro Cuatrecasas

Department of Molecular Biology, The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Image intensified fluorescence microscopy has been very useful in visualising the patterns and mobility of receptors for epidermal growth factor (EGF), insulin and α_2 -macroglobulin in intact cells^{1,2}. Recently, we have synthesised a bioactive derivative of enkephalin³, Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine, and used it for the microscopic visualisation and localisation of opiate (enkephalin) receptors in neuroblastoma cells⁴. In contrast to the case of polypeptide hormones^{1,2}, where fluorescently labelled receptors are initially distributed uniformly and quickly form patches which are subsequently internalised^{1,2}, the enkephalin-labelled receptor patches in neuroblastoma cells appear only slowly and are not internalised⁴. Sulphydryl groups are involved in the binding of opiates to brain membranes and may affect differentially the binding of agonists and antagonists^{5,6}. Also, sulphydryl and disulphide groups are involved in the binding sites of adrenergic, cholinergic and muscarinic receptors⁷⁻¹¹. These observations prompted us to examine the effects of disulphide and sulphydryl reagents on the clustering of opiate (enkephalin) receptors in N4TG1 neuroblastoma cells. We report here that in these cells there are reactive sulphydryl and disulphide groups which are essential for cluster formation (but not binding), and that a sulphydryl-disulphide exchange reaction may be involved in this process. In addition, the sulphydryl reagents seem to dissociate the two steps of binding and cluster formation, and thus provide a tool for studying the pharmacological importance of receptor clustering.

When N4TG1 neuroblastoma cells are incubated with 10⁻⁸ M of a rhodamine-labelled enkephalin derivative (30–40 min at 25 °C), many patch-type areas of bright fluorescence, spreading over the cell surface, are detected (Fig. 1a). These patches do not internalise⁴ with longer periods of incubation at 25 °C or 37 °C. Furthermore, most of the fluorescent patches disappear and more than 95% of the cell-bound radioactively labelled enkephalin dissociates rapidly as intact peptide if the cells are washed⁴. The clusters are not seen in the presence of 10⁻⁶ M [D-Ala², D-Leu⁵] enkephalin (Fig. 1b). Incubation with specific sulphydryl reagents (10⁻³ M iodoacetate, 10⁻⁴ M iodoacetamide, 10⁻³ M *N*-ethylmaleimide or 10⁻³ M 5,5'-dithiobis(2-nitrobenzoic acid)) in conditions identical to those used for binding assays (Table 1) result in the uniform distribution of the fluorescently labelled receptors (Fig. 1c); fluorescence can be displaced (inhibited) by 10⁻⁶ M [D-Ala², D-Leu⁵] enkephalin. This pattern is not affected by further oxidation with 10⁻⁴ M hydrogen peroxide. Identical results are obtained when the cells are pretreated with 10⁻⁵ M dithiothreitol, washed and treated with sulphydryl-modifying reagent, in the presence or absence of subsequent oxidation (data not shown). Pretreatment or incubation in the presence of 10⁻⁴ M hydrogen peroxide alone does not prevent cluster formation. The diffuse patterns observed are probably due to the modification of free sulphydryl groups and not to a reduction in the number of binding sites, as in these conditions the cells retain their ability to bind [¹²⁵I]-[D-Ala², D-Leu⁵] enkephalin (Table 1).

When the rhodamine-enkephalin derivative is added to the cells in the presence of 10⁻⁵ M dithiothreitol, no patches are seen and the fluorescence is distributed evenly over the cell surface (Fig. 1d), even after prolonged (2 h) incubation at 25 °C. This diffuse pattern (10⁻⁵ M dithiothreitol, 40 min) is quickly

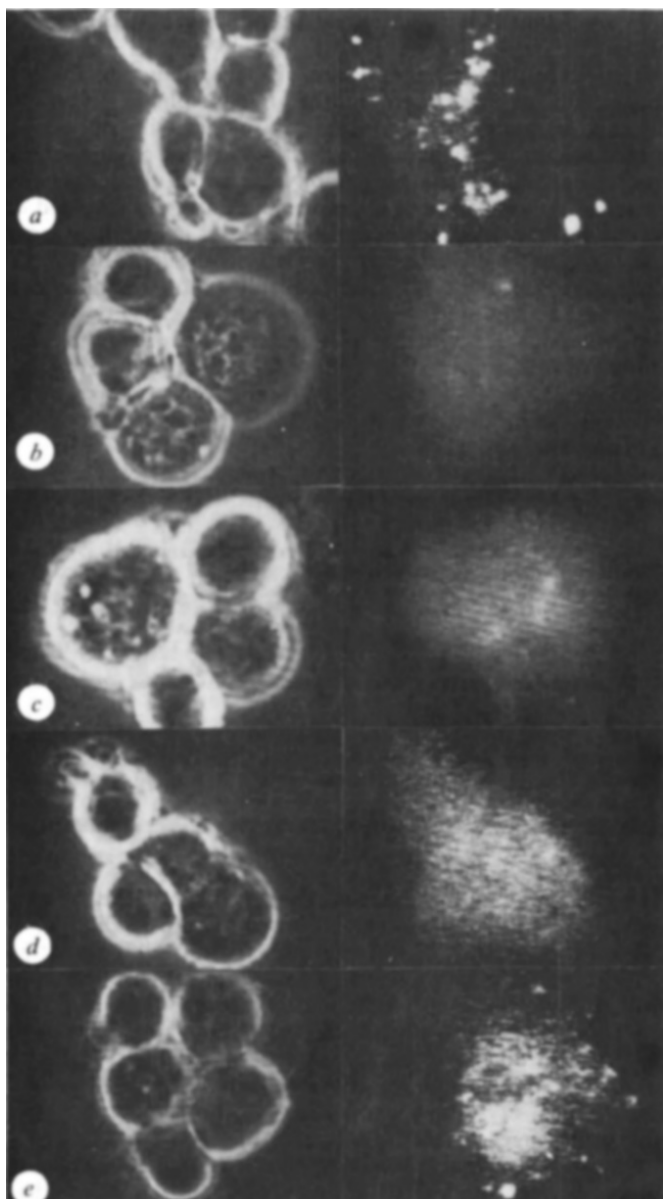


Fig. 1 Fluorescent visualisation of rhodamine-enkephalin binding to N4TG1 cells. On the left, phase micrographs $\times 865$ on the right, fluorescence micrographs of the same field. *a*, The cells were incubated for 40 min at 25°C with 10^{-8} M rhodamine derivative; *b*, incubation as in *a* but in the presence of 10^{-6} M [D-Ala², D-Leu⁵] enkephalin; *c*, incubation (identical conditions as for binding assays in Table 1) with 10^{-3} M iodoacetate; *d*, incubation with 10^{-5} M dithiothreitol; *e*, pretreatment with 10^{-5} M dithiothreitol (30 min) followed by addition (for 10 min) of 10^{-4} M H₂O₂. Identical results to that shown in *c* were obtained with 10^{-4} M iodoacetamide, 10^{-5} M *N*-ethylmaleimide and 10^{-3} M 5,5'-dithiobis(2-nitrobenzoic acid). The fluorescence was recorded on videotape using a silicon intensified target television camera⁴, and photographs were taken from the video monitor. The cells were not fixed for these studies.

(10–15 min) modified to one of clustering by oxidation with 10^{-4} M hydrogen peroxide (after 10 min, Fig. 1*e*; after 15 min it is similar to that seen in Fig. 1*a*). Clusters reappear spontaneously but slowly (about 75 min) when the cells are pretreated for 20 min with 10^{-5} M dithiothreitol, washed and incubated at 25°C in the presence of the fluorescent peptide. However, the size, brightness and number of clusters are reduced (data not shown) compared with the control (Fig. 1*a*) or with hydrogen peroxide-induced oxidation (Fig. 1*d*).

The clusters, once formed, cannot be dispersed by the subsequent addition of 10^{-3} M iodoacetate, 10^{-4} M

iodoacetamide or 10^{-3} M dithiothreitol, suggesting that the sulphhydryl and disulphide groups are protected when the hormone is bound to the receptor and clusters are formed. The clusters can be dispersed by the addition of 100 mM sodium, which also markedly reduces the binding of the peptide, or by the simultaneous addition of 10^{-3} M dithiothreitol and 100 mM sodium. These results are consistent with previous studies with brain membrane preparations^{5,6} which suggest that sulphhydryl groups are associated with the opiate receptor and that binding of the hormone reduces their reactivity with sulphhydryl-alkylating reagents.

Table 1 Effect of sulphhydryl, disulphide and miscellaneous reagents on [¹²⁵I]-[D-Ala², D-Leu⁵] enkephalin binding and clustering in N4TG1 cells

Reagent	¹²⁵ I-[D-Ala ² , D-Leu ⁵]-enkephalin specific binding (% control)	Hormone-receptor complex pattern
Iodoacetate		
0.1 mM	100	Few clusters
1 mM	89	Uniform distribution
10 mM	83	Uniform distribution
Iodoacetamide		
0.01 mM	95	Few clusters
0.1 mM	89	Uniform distribution
1 mM	87	Uniform distribution
<i>N</i> -ethylmaleimide		
0.01 mM	95	Uniform distribution
0.1 mM	50	Uniform distribution
1 mM	28	—
Dithiothreitol		
0.01 mM	94	Uniform distribution
0.1 mM	83	Uniform distribution
1 mM	77	Uniform distribution
Hydrogen peroxide		
0.1 mM	100	Clusters
Dithiothreitol (0.01 mM, 30 min) + hydrogen peroxide (0.1 mM)	95	Clusters
5,5'-Dithiobis(2-nitrobenzoic acid)		
0.1 mM	85	Clusters
1 mM	65	Uniform distribution
Sodium azide		
1 mM	82	Clusters
10 mM	67	Clusters
Dinitrophenol		
0.5 mM	100	Clusters
1 mM	98	Clusters
Methylamine hydrochloride		
1 mM	81	Clusters
10 mM	76	Small reduction in the number of clusters
30 mM	52	Few clusters

N4TG1 neuroblastoma cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. At confluence, the cells were removed from the flask by incubation with 1 mM EDTA for 5 min at 37°C. The detached cells were washed three times with 25 mM Tris-HCl, pH 7.7, containing 0.25 M sucrose, 2 mM MgCl₂ and 5 mM glucose, and finally suspended in the same buffer. Fluorescence microscopy was done as described in Fig. 1 legend. Binding assays: the radioactive enkephalin (0.5 nM, specific activity 2 Ci μ mol⁻¹) was incubated with different concentrations of the reagent at 25°C for 60 min in a final volume of 0.25 ml containing N4TG1 cells (4×10^6 cells ml⁻¹). When iodoacetate and iodoacetamide were tested the cells were pretreated with the reagent for 15 min at 25°C, washed by centrifugation and the binding measured by filtration under vacuum through Whatman GF/C filters as described previously^{14–16}. Specific binding represents the bound radioactivity which can be competed for by 10^{-6} M unlabelled [D-Ala², D-Leu⁵] enkephalin; each value is the mean of duplicate incubations which varied by less than 5%. All reagents were dissolved immediately before use.

To investigate further the mechanisms involved in clustering we examined the effects of sodium azide (1 and 10 mM) and dinitrophenol (0.5 and 1 mM), as shown in Table 1. The results suggest that the formation of clusters, in these conditions, is independent of the generation of metabolic energy. The effects of methylamine on clustering were also examined as this reagent has been found to inhibit the clustering of α_2 -macroglobulin and EGF on the fibroblast cell surface¹². It was suggested¹² that certain amines may act by increasing the intracellular pH, thus possibly interfering with protein-protein interactions necessary for clustering, or alternatively by inhibiting a calcium-requiring transglutaminase enzyme which may be involved in cluster formation. No inhibition of clustering is seen with 1 mM methylamine whereas with 10 mM there is a small reduction in the number of clusters. At 30 mM methylamine only a few clusters are observed but the binding is substantially reduced (52%). Moreover, in contrast to the observations with α_2 -macroglobulin and EGF, in which clusters are not formed in the absence of extracellular calcium, the clusters in neuroblastoma

cells are formed in the absence or presence of calcium or EDTA⁴.

It is interesting that the nature of the binding and surface distribution of the neuromodulator, enkephalin, is quite different from that of polypeptide hormones with respect to internalisation^{1,2,4,13} and the effects of sodium and calcium⁴. Our data strongly suggest that both sulphhydryl and disulphide groups are specifically involved in the molecular processes involved in the aggregation of ligand-receptor complexes in neuroblastoma cells. Whether the clusters are formed or stabilised by simple sulphhydryl-disulphide interchange reactions, or whether these functional groups are involved in some other capacity such as transglutaminase¹², remains to be established. However, prevention of cluster formation without affecting receptor binding may provide a tool for studying the roles of receptor occupancy and subsequent clustering in such biological effects as opiate tolerance and addiction.

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Relationship between crystallin mRNA expression in retina cells and their capacity to re-differentiate into lens cells

R. M. Clayton*, I. Thomson† & D. I. de Pomerai‡

* Institute of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh, UK

† Napier College of Commerce and Technology, Colinton Road, Edinburgh, UK

‡ Department of Zoology, University of Nottingham, University Park, Nottingham, UK

Differentiating vertebrate embryo tissues pass through a stage when they have the potential for several pathways of differentiation, some of them revealed only by experimental manoeuvres. As differentiation proceeds, the potential for alternative pathways becomes progressively restricted and is eventually lost. We have investigated the molecular basis for this phenomenon, using the chick embryo retina, which has a potential for differentiation into lens cells. This potential requires special conditions for its manifestation; it declines during development and is finally lost before hatching. Lens protein (crystallin) mRNA is expressed in early embryo retina: its levels decline in parallel with the decline in lens-forming potential. There is also evidence of an ontogenic change in post-transcriptional regulation. Such a relationship between heterologous mRNA and heterologous differentiation potential may be of general application.

In the vertebrate eye the neural retina (NR) and the pigmented epithelium (PE) of the retina are of different developmental origin to the lens, but embryonic cells from both tissues are able to redirect their differentiation in cell culture into lens fibre cells, with characteristic ultrastructure, and containing the characteristic lens proteins, the crystallins. This phenomenon has been termed transdifferentiation: it has been found in all vertebrates so far tested¹⁻³. In the chick, which has been the

most intensively studied example, the expression of the capacity for transdifferentiation is regulable by cell culture conditions^{1,3-5} and depends on the age of the embryo, transdifferentiation being more frequent and more rapid from retina of early as compared to later embryos, while the relative balance of the crystallins synthesised is also affected⁶⁻⁸. The three classes of crystallins in birds are the α -, β -, and σ -crystallins. Lentoids (structures composed of lens fibre cells) have the highest proportions of σ -crystallins when derived from retinas of earlier embryonic stages (Table 1).

We have found that crystallin mRNAs are expressed in 8-day embryo neural retina (NR) at levels of about 0.025% and in 8-day embryo retinal pigmented epithelium (PE) at levels of about 0.04% of those found in 1-day-old lens, but embryo muscle and headless embryos contain no detectable crystallin mRNAs as measured by hybridisation to crystallin-specific cDNA⁹. During the process of transdifferentiation into lens from 8-day embryo NR or PE, the levels of crystallin mRNA rise, and in terminally transdifferentiated NR cultures, reach between 14% and 17% of the levels found in 1-day-old lens. Terminally transdifferentiated PE cultures reach 1.25% of the level in day old lens^{1,10,11}.

Since the rate and frequency of transdifferentiation from 3½-day embryo NR is 2-3 times higher than from 8-day embryo NR, we tested the hypothesis that the levels of crystallin mRNA expressed in the earlier stages will be higher than in the later stages.

The hybridisation data (Fig. 1) show that 3½-day embryo eye cups (future NR and PE) express crystallin mRNAs at about 10 times the level found in 8-day embryo NR, while none can be detected in the retina of 1-day-old chicks, by which stage transdifferentiation into lens is no longer possible (ref. 8 and Table 1). Paradoxically, although low levels of crystallin protein are detectable in 8-day embryo NR¹², we have not detected crystallins in 3½-day eye cups, suggesting possible ontogenic changes in the regulation or the translatability of the mRNA.

Evidence pointing to post-transcriptional sequestration for some β -crystallin mRNA has been reported for day-old chick lens¹³. It remains to be tested whether sequestration of crystallin

Table 1 Transdifferentiation into lens cells from chick embryo neural retina

	Age of embryo (days): 3½	6	8	12	15	17	19	(1-day-old chick)
Time for lentoid formation, days	8–15*	23†	19–25†	25†	28–35†	50†	—†	—†
% of lentoid clones formed	20*		8*	Few*	Few*	—*	—*	
Multipotential clones	+	Few*	Rare*					
σ-crystallin in lentoids, %	80*	50†	25†	6†	3†	0†	—†	—†
σ:α crystallin ratio	20:1*	7:1†	3:1†	2:1†	2:1†	α, β only†	—†	—†
Crystallin mRNA compared to day old lens	0.18%		0.025%‡					ND
Final level of crystallin mRNA obtained in terminal culture compared to 1-day-old lens	17%§		14%‡					
Time required	3 weeks§		6 weeks‡					
Crystallin, as percentage of total soluble protein¶	α ND β ND σ ND		0.015% 0.04% ND					0.04% 0.13% ND

ND, not detectable (<0.01% for all cases except δ-crystallin, <0.005%).

* From refs 3, 6, 7.

† From ref. 8.

‡ From refs 9–11.

§ From refs 13, 14.

|| This work.

¶ Haemagglutination inhibition assay. Limits of detection for antisera used: α, 0.01%; β, 0.01%; σ 0.005%.

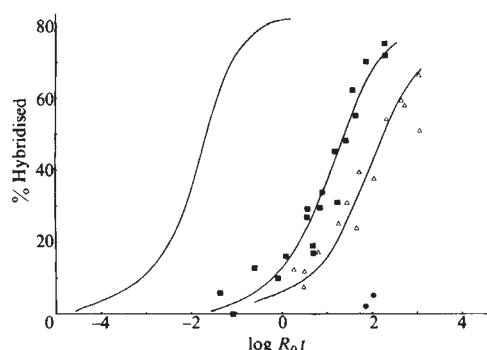


Fig. 1 Lenses from 500 3½-day-old chick embryos were removed, checked for their integrity and discarded to eliminate all possibility of contamination of eye cups by fragments of lens. The eye cups were then excised and stored in liquid nitrogen. Cytoplasmic mRNA and soluble protein were prepared from two separate such aliquots^{10–12}. Neural retina from 100 8-day embryos and retina from 50 day-old hatched chicks was similarly processed. The protein fraction from each sample was analysed by haemagglutination inhibition assay using antisera to crystallins¹². Total cytoplasmic RNA was prepared from each of these tissues by first homogenising in 0.35M sucrose, 10 mM Tris pH 7.5, 50 mM KCl, 1.5 mM MgCl₂, followed by centrifugation at 10,000g for 10 min at 4°C. RNA was extracted by addition of an equal volume of phenol/chloroform (1:1 by volume), 0.05% 8-hydroxyquinoline, and collected by ethanol precipitation. Poly(A)-containing RNA was fractionated by two cycles of oligo(dT)-cellulose chromatography, and collected by ethanol precipitation as before. Complementary DNA (cDNA) was synthesised from 1-day-old lens⁹. mRNA at 100 μg ml⁻¹ was added to a 100 μl incubation mix containing 10 μg ml⁻¹ oligo(dT)_{12–18}, 75 μg ml⁻¹ actinomycin D, 375 μg ml⁻¹ bovine serum albumin, 50 mM Tris-HCl pH 8.2, 5 mM Mg acetate, 50 mM NaCl, 2 mM dithiothreitol, dATP, dGTP, and dTTP (each at 400 μM) and 45 μM ³H-dCTP (20 Ci mmol⁻¹). Abundant cDNA (complementary only to the crystallin mRNA) was prepared by two cycles of hybridisation to lens mRNA to a R₀t value allowing only hybridisation of the most abundant sequences⁹. Appropriate volumes of mRNA and cDNA solutions in sterile distilled water were mixed, lyophilised and redissolved in 0.5 M NaCl, 25 mM HEPES pH 6.8, 0.5 mM EDTA, and 50% (v/v) deionised formamide. Aliquots were heated to 60°C for 5 min, and allowed to hybridise at 43°C for various times. The proportion of cDNA in the hybrid was determined by resistance to treatment with S₁ nuclease⁹. ■, 3½-day embryo eye cup mRNA; ●, 1-day-old chick retina mRNA; △, 8-day embryo neural retina mRNA. For comparison, the solid line to the left shows values for 1-day-old lens mRNA (ref. 9).

mRNAs or instability of the crystallin proteins characterise the 3½-day eye cup.

The presence of low levels of crystallins in the freshly excised retina of day old chicks in the absence of detectable crystallin mRNA suggests that those crystallins which are synthesised during the embryonic period are not broken down.

Measurement of hybridisable crystallin mRNA in transdifferentiating 3½-day embryo NR cultures shows that the levels attained in terminally differentiated cultures are similar to those achieved by 8-day embryo NR but these levels are reached by 3 weeks from the 3½-day embryo^{13,14} as compared to 6 weeks for 8-day embryo NR. These data, and the data presented here, suggest that mRNA and transdifferentiation potential are related, but we have not yet determined whether the change affects the numbers of cells expressing crystallin mRNA or the levels per cell. This is currently under investigation. Previous data have, however, indicated that some of the cellular conditions and manipulations favouring transdifferentiation exclude cell selection as a possible mechanism^{1,4}. Many mRNAs normally coexist in cells, so it seems possible that the ease of differentiation along a particular pathway has a molecular basis in the relative levels of the mRNA appropriate to the end point in that pathway.

If low levels of crystallin mRNA are the preconditions for transdifferentiation into lens, (as we propose here), then cell dissociation followed by reassociation are the permissive conditions, and the directive conditions include cell culture medium, cell density, embryonic age and genotype, which all affect both the direction of transdifferentiation and the relative balance of the different crystallins ultimately synthesised. Evidence for these latter propositions is assembled and discussed more fully elsewhere¹.

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A double-stranded β -helix with antiparallel chains in a crystalline oligo-L-D-peptide

E. Benedetti, B. Di Blasio & C. Pedone

Istituto Chimico, Università di Napoli, Via Mezzocannone 4, 80134 Napoli, Italy

G. P. Lorenzi, L. Tomasic & V. Gramlich

Technisch-Chemisches Laboratorium, ETH-Zentrum, 8092 Zurich, Switzerland

There is much interest in the ionophore-mediated and channel transmembrane transport of alkali metal ions. Valinomycin and gramicidin A, which is a linear pentadecapeptide with alternating L-D-residues, are classic representatives of carrier and channel-type ionophores, respectively, and their structure-function relationships are being intensively studied¹⁻⁴. Helical structures for alternating D,L peptides have been proposed from conformational energy calculations⁵⁻⁷, and from experimental spectroscopic data single-helical structures have been postulated for gramicidin A (ref. 8). Stereochemical and energy considerations^{7,9} have suggested that polypeptides with alternating L- and D-amino acid residues may take up coaxial double-helical β -structures, both parallel and antiparallel, which are stabilised by systematic interchain NH...OC hydrogen bonds. Experimental results supporting the occurrence of structures of this kind have been obtained by Veatch, Fossel and Blout² for gramicidin A in nonpolar solvents, and by Lotz, Colonna-Cesari, Heitz and Spach¹⁰ for poly-(γ -benzyl-D,L-glutamate) in the solid state. However, there has previously been no experimental determination of the detailed structural and conformational parameters characterising these structures. We now provide detailed conformational parameters of a double-stranded β -helix with antiparallel chains obtained by single crystal X-ray analysis of the model L-D peptide Boc-(L-Val-D-Val)₄-OMe (ref. 11).

The compound investigated crystallises from ethyl acetate solutions in the trigonal P3₂21 space group with cell dimensions $a = 12.760$ Å, $c = 63.190$ Å and $d_{\text{calc.}} = 1.05$ g cm⁻³, based on monohydrated units (C₄₆H₈₄N₈O₁₁·H₂O) and $Z = 6$. Data, collected on the Enraf-Nonius CAD-4 PDP 11/34 diffractometric system of the Centro di Metodologie Chimico Fisiche at the University of Naples, consisted of 6,399 measured reflections, 2,721 of which had a net intensity $> 3.0\sigma$ (I). The structure was solved using Multan and has been refined by a least

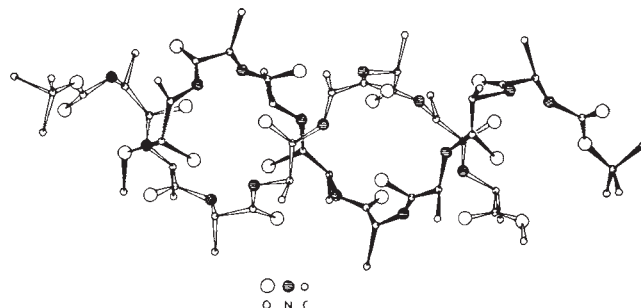


Fig. 2 The double-helical dimer as viewed along the b axis. The side chains are omitted from the model for clarity.

squares procedure, with isotropic thermal parameters for all atoms to an R factor of 0.13 for the observed reflections.

Figure 1 shows the molecular model of the octapeptide chain. Its structure consists of dimers (Fig. 2) in which each peptide chain is related to the other by a crystallographic twofold axis perpendicular to the helix axis, giving rise to a double-stranded, left-handed helix of antiparallel chains. Seven independent (14 in one dimer) NH...OC intermolecular hydrogen bonds link two antiparallel chains, with N...O distances of 2.7–3.0 Å. Only one potential hydrogen bond donor per chain is not involved in hydrogen bonding. The conformation of each chain can be described by the φ and ψ conformational parameters. They assume values in the range of $-148^\circ \leq \varphi \leq -112^\circ$ and $98^\circ \leq \psi \leq 122^\circ$ for the L-residues and $80^\circ \leq \varphi \leq 129^\circ$ and $-163^\circ \leq \psi \leq -152^\circ$ for the D-residues. Side chains assume different conformations for L- and D-residues; all four D-residues have the two methyl groups of the isopropyl moiety in g_+ and g_- conformations (close to $+60^\circ$ and -60°) with respect to the N atom, whereas in L-residues the g_+ , t conformation ($+60^\circ$, 180°) is observed three times and the g_- , t conformation (-60° , 180°) only once. Along the axis of each dimer four distinct sections (about 4 Å apart) can be seen, in each of which four carbonyl oxygen atoms lying in a non-planar arrangement, could be available for possible coordination to metal ions within the core of the chain. The distance of each carbonyl oxygen from the chain axis is 2.5–2.9 Å. Projections along the dimer axis clearly show the steric hindrance produced by the N- and C-terminal blocking groups which partially close the mouth of the 'cylinder'. The water molecule is hydrogen bonded to the urethane carbonyl oxygen and a symmetry-related water molecule. All peptide linkages, as well as the urethane bond, occur in the *trans* configuration ($\omega = 180$), with maximum $|\Delta\omega| \leq 6$.

The dimer can thus be regarded as a cylinder with an hydrophilic inner core of almost planar peptide bonds nearly parallel to the chain axis, and an hydrophobic outer layer of isopropyl groups of the valyl residues. The length (from N₁ to C₈) and average inner diameter of the cylinder are 14.8 Å and 5.1 Å, respectively.

The double-helical structure of the dimer described here can be considered a stereochemical model of the antiparallel β -double helix proposed by Blout and coworkers³ for species 3 of gramicidin A.

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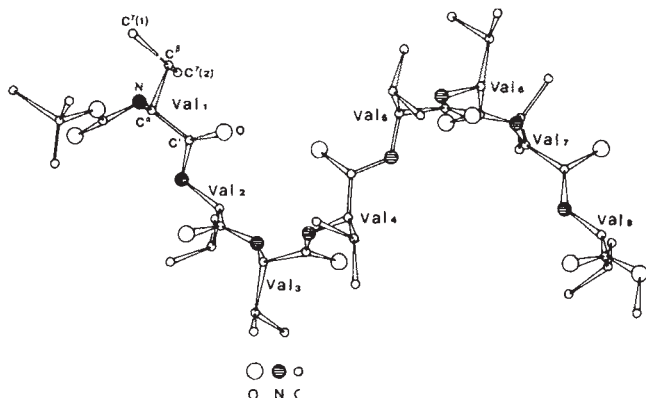


Fig. 1 Molecular model of Boc-(L-Val-D-Val)₄-OMe as viewed along the b axis. The eight valine residues are schematically indicated; only the first is labelled.

CHRISTMAS BOOKS

The guiding star

W. H. McCrea

The Star of Bethlehem Mystery. By David Hughes. Pp. 218. (Dent: London, 1979.) £7.95.

THE story of the star of Bethlehem comes to us in the vivid words of Matthew 2: 1-12, and nowhere else in the *Bible*. The mystery in the title is that of the star's identity. If a disproportionate part of this review is taken by the reviewer's response to the challenge of David Hughes' interpretation, it is in tribute to the fascination of the book. That interpretation is as follows:

The year 7 BC saw the rare occurrence of a triple conjunction of the planets Jupiter and Saturn. As its most relevant consequence, it caused these planets to keep about one degree apart in the sky from mid-September to early December. The planet-pair during that interval was the 'star' of Bethlehem. In extant cuneiform texts, Babylonian astronomers had accurately predicted its observable behaviour. The Magi of the story were Zoroastrian priests schooled in such astronomy. At the time they shared a widespread expectation of the manifestation of a Messiah. Their sophisticated astrology made them take some — predicted or observed — feature of the behaviour to augur this Messiah's birth and to denote that it would occur amongst the Jewish people in the land of Palestine. Such was their conviction that a group set out to discover the child. They made for Jerusalem as the chief city of Palestine; there they created a stir by asking about a new-born 'king'. In consequence, King Herod directed them to Bethlehem. The 'star' reappeared, and somehow indicated their goal. There the Magi paid joyful tribute, and offered gifts which they had dedicated to this consummation.

Ruthlessly distilled, such is the account emerging from Hughes' comprehensive presentation of the astronomical and historical evidence. His astronomical conclusions are generally wholly convincing. However, there are difficulties in the account as a whole. First, as we shall see, it explains too much. Then, although Hughes has convinced me that he could have convinced the Magi that their own conventions would produce the suggested astrological prediction, there is no known evidence that the Magi made this prediction. Again, by inadvertently implying such a success for astrology, the account apparently resolves one mystery by creating a worse mystery.

Let us consider an alternative approach, and let us do so with reference to the

version of the Matthew story in the *New English Bible*. The quoted passage is succinct; let us be the same. We proceed on the basis — to be tested — that it is historical. Matthew could not know the story from personal experience. He introduces the astrologers (as the Magi become in *NEB*) simply as "arriving" in Jerusalem. There is no suggestion that a star induced them to come, none that it guided them. So they came as ordinary visitors. We know there were periodical visits by "inhabitants of Mesopotamia" (Acts 2:9) and the like; we infer that the astrologers were amongst such visitors. But this particular visit turned out to be uniquely eventful; the homecoming participants would rehearse the tale until it became a set piece. Other visitors would carry it back to Jerusalem, and Matthew has passed it on to us. In confirmation, linguists remark how each mention of the star is in terms that astronomer/astrologists would use. Also, the passage is singular in that Matthew finds no prophecy to quote about the star. If the account is historical, we are thus most probably dealing with the astrologers' version.

When the account opens, it all but explicitly admits that the astrologers then knew about the birth of Jesus as something out of the ordinary. So they would cast about for a portent. An observed acronychal rising of a 'star' supplied it. Portents belonged to rulers, so the child was 'born to be king' — of the Jews, for he was in Judea. In spreading this notion, we cannot know how much the astrologers themselves believed. Anyhow it upset King Herod, who would tolerate no threat to his dynastic obsession. He asked his advisers about the birthplace of "the Messiah" — presumably trying to conceal his motive by not saying "king". Since Matthew has them replying in the identical words in which he had just recorded the birth of Jesus, he appears to tell us that they had knowledge of the birth; but they covered up by "referring" Herod to a prophecy about Bethlehem. He surreptitiously met the astrologers, interrogated them about the star, despatched them to Bethlehem, and ordered them to report back.

In Bethlehem, the astrologers found the "place where the child lay", and soon they "entered the house". In the intervening moments "they were overjoyed at the sight of the star . . . which they had seen at its rising". It was early evening because, although the star was visible, the hour was suitable for the strangers to visit the child and his mother. So the star had just "come

out", and it could not have guided them on the way. Matthew does not say it did, but simply that it was there ahead of them. Granting that it was a real star, the sight would have been the same that evening anywhere else in Palestine, and much the same any other evening within days. But to astrologers a star would be of interest only if doing something special — like rising acronychally; or seen at a special moment — like this, when their pretensions were on trial. The astonishing sight at this moment of the self-same star that they had adopted as portent seemed miraculous confirmation of their claims, no matter how little faith they had in them — hence their rapture. So they entered, "saw the child with Mary his mother" and saluted him. The record says, "then they opened their treasures"; that they had to do this, between the salutation and offering gifts, implies that the turn of events had taken them by surprise. Then they went home; and that is the end of what we may take to be their own story.

Matthew's continuation shows that the astrologers evidently told Joseph of their intention to evade Herod. The simplest conclusion for Herod would have been that they had failed in their mission. His murderous reaction indicates that some rumour of their finding the child got round to him.

This narrative seems to overcome the difficulties found in Hughes'. It purports to account for nothing beyond what Matthew tells us. Although it takes account of the behaviour of astrologers as such, nothing depends upon any interpretation on their part beyond what is directly implied by the text as we have it, and nothing implies any success or benefit attributable to astrological prediction. The climax of Matthew's account of the astrologers in the slaughter of the Innocents is indeed a terrible indictment of their activities, as well as of Herod.

Certain possible difficulties mentioned by Hughes himself are also resolved. For instance, the fact that two and only two sightings of the star play any part is automatically explained. Also, as the first sighting is here held to have had nothing to do with the astrologers setting out for Jerusalem, it could have occurred any time up to their arrival; so it denotes no constraint upon the duration of their travels.

On the revised account, Babylonian astrological conventions carry less weight in attempts to identify the star. Nevertheless, purely astronomical

considerations, and elementary regard for what could interest astrologers, still seem almost overwhelmingly to support the identification with the planet-pair Jupiter and Saturn favoured by Hughes. Or it might be that 'the' star was just Jupiter, with the attendant Saturn making it more than usually noticeable or interesting for the time being.

The fact that the account can be so readily related to a known astronomical phenomenon, and its remarkable internal consistency, are strong evidence for its historicity. If both lines of evidence are accepted, they indicate that the Magi/astrologers went to Bethlehem in 7 BC sometime after mid-September, as Hughes would agree, but some of his other time-constraints may be relaxed somewhat.

It was David Hughes himself who, by his article in *Nature* (264, 513-517; 1976) on this most famous star of all, initiated the recent renewed interest of, particularly British, astronomers in the subject. In his book, he now surveys it in the widest possible setting. He reviews the results of an enormous amount of published scholarship in biblical and historical studies, in archaeology and anthropology of the Middle East, and in astronomy itself. Naturally, he takes full account of the discussions instigated by his *Nature* article. He has devoted acute critical and constructive thought to collating this vast material, with most illuminating results. He re-discusses probably every serious suggestion that has ever been advanced to account for the 'star' by astronomical phenomena or other natural processes. He writes with vigour and clarity, reinforced by numerous well-conceived tables and diagrams, happily-chosen plates, and a full bibliography. The wealth of background and sequential material is bound to make it harder to trace the central theme of the identity of the star and what it can tell about the birth of Jesus. However, most readers will be fascinated by it, as well as by this theme itself.

The planetary conjunction hypothesis regarding the star goes back at least as far as Kepler. A leading modern exponent, particularly in regard to its setting in Babylonian astronomy is K. Ferrari d'Occhieppo, to whose valuable monograph *Der Stern der Weisen* (Vienna, 1968, second edition 1977) Hughes makes frequent reference. Hughes' book is the only full account in English, and I think it is the most comprehensive work by any astronomer on the subject of the 'star'.

The book inspires yet another line of thought. Questions of significance will be discussed for evermore. But, wherever possible, matters of simple fact should be settled once and for all. In regard to an important date, for example, it ought now to be possible to use a computer to sift all the evidence, test it for compatibility and so forth, and in at least some cases produce a uniquely acceptable solution. In other

cases a computer might determine the date by predicting testable consequences of all reasonable hypotheses about it. The possibilities seem unbounded. □

Biological analogy

Martin Pollock

The Evolution of Designs: Biological Analogy in Architecture and the Applied Arts. By P. Steadman. Pp. 276. (Cambridge University Press: Cambridge and New York, 1979.) £9.50.

THIS book is essentially an analytical description of the evolution of design in the construction of human artefacts, being focused, as the subtitle indicates, on architecture and the significance of biological analogy in attempts to understand the principles involved.

The biological analogy is, indeed, a fertile and stimulating one and there is plenty of interest in how Professor Steadman pursues, criticises and finally rejects it as "false". Close comparisons between living organisms and man-made structures can be drawn at many different levels: the whole system designed "for a purpose"; classification of different but related types; structural anatomy (endo- and exoskeletons in both cases); ecology (form related to function and the environment); evolution (Darwinian versus Lamarckian); ornamentation (vestigial organs in plants and animals and so-called "skeuomorphs" in architecture that involve conversion of originally necessary features into purely decorative patterns); and many others, all considered in some detail.

During the course of this comparison, the author discusses various attempts to develop a general understanding of the principles of design over the past 150 years. Amongst these, particular critical attention has been paid to the general evolutionary doctrines of Herbert Spencer, to the fascinating "Purism" of Le Corbusier and Ozenfant, whose views on the basic banal forms they called "objets-types" (such as the bottle, tobacco-pipe, drinking glass, and so on) which, having completed their evolution to combine maximum utility with maximum manufacturing economy and acceptability (including aesthetic appreciation), correspond closely to Darwinian principles applied to artefacts; and, in more recent times, to Christopher Alexander whose views on the "unselfconsciousness" of the better designs in architecture are presented as being close to the biological analogy; and to David Clarke's *Analytical Architecture* (1968), which also argues against cultural systems having a "life of their own", considering them rather as part of biology, comprising "information systems of cumulatively

W. H. McCrea is Emeritus Professor of Theoretical Astronomy at the University of Sussex, Brighton, UK.

acquired knowledge, partly replacing instinctive behaviour in man and selectively advantageous in his struggle for survival" (page 220).

All this is of great interest and value. But the style is rather heavy and the presentation somewhat confused — perhaps even misleading at times — largely because of this concentration on the significance, or otherwise, of a biological analogy. Indeed, the concept itself of *analogy* (defined in the *Oxford English Dictionary* as "resemblance of form or function without identity of essence") differs according to taste and habit. Sometimes it is simply a question of similarity without identity: for instance, most buildings, like many organisms, need light and insulation and internal structural support; they are inevitably subject to the same chemical, physical and mechanical forces of their environment and must therefore have features in common. A very different type of analogy is primarily anthropomorphic — sometimes to the point of being poetic fancy, such as the resemblance of the façade of a house to a human face. After all, man is always constructing models of what he perceives; and because he finds it hard to have totally new ideas, he is always making comparisons and extensions, usually with reference to human experiences already encountered. He will see himself and familiar objects in almost anything. To pay too much attention to analogy (in all its varied forms) in order either to discard or accept any particular interpretation can therefore be misleading. Its value, I would argue, is in helping to visualise alternative ways of approaching an understanding of a problem.

Philip Steadman rejects the "biological analogy" largely because the evolution of design, like other forms of cultural expression, is Lamarckian (that is, directly moulded by the environment) rather than Darwinian (due to selection of a very few types out of a huge number of possible variants) which is the currently accepted theory of organic evolution. Even here he admits that the situation is not perhaps as clear cut as might at first appear: successful designs *do* survive through some sort of selection, from a large variety, by human choice which acts as the controlling environment as well as the apparently direct source of the various designs. But most biologists would agree that cultural evolution, whatever its mechanism, does not involve the permanent stable change in DNA composition that occurs in the evolution of living organisms themselves. And Popper is quoted (page 216) as pointing out that if all literature and other types of artefact were totally destroyed, the

human race would have to start again almost from scratch. Only memory would remain and that would persist through generations only in so far as verbal transfer of information from one person to another continued. But we really know very little about human evolution over the last 50,000 years — that is, during the period when man himself has been modifying his own environment and so possibly directly affecting the manner in which the environment has been playing a part in his evolution, at least to an extent far greater than have other organisms.

This only emphasises another problem to be faced in the exploitation of biological analogy; namely the importance (and difficulty) of getting the biological situation right in the first place.

A simple example of this would, indeed, be Steadman's second argument for rejecting the Darwinian analogy (page 101) which is that cultural evolution is "reticulate"; that is, it combines elements from different origins and is therefore fundamentally distinct from the Darwinian "tree" in which the branches always diverge and never rejoin. Well-versed in biology generally, the author is not here up-to-date, as possible instances of "reticulate" evolution in biology are becoming more and more convincing and the prokaryotic origin of both the chloroplasts and mitochondria of eukaryotes is now generally accepted: a rather pretty analogy with (for instance) the common practice in early Islamic architecture of actually using (not just copying) pilfered Roman pillars for decorative support of vaulting such as that of the famous Omayyad mosque in Damascus.

But this is really neither here nor there in the study of the evolution of design generally. Reference to analogy is useful for hanging a discussion upon but, in the last resort, the relevant processes have to be analysed in terms of the direct influences that operate. And the problem centres on the extent to which man's activities can be considered to be simply one aspect of the way some organisms operate: strictly a problem in biology, complicated though that may be. The opposite approach, favoured by Professor Steadman, is that man's cultural expression must be studied, at least some extent, in its own right: there is such a thing as design *per se*; it is the result of conscious mental activity and cannot be reduced by scientific dissection to analysis in strictly biological terms. And from that angle one is plunged (if not explicitly) into all the deep conundra that have plagued philosophers from time immemorial: determinism, intrinsic free-will, reductionism, dualism, and so on.

At the end, the author asks the question: "What are the features or properties of artefacts of all kinds to which scientific study should be directed and about which scientific prediction might be made?" (page 238). To the question: "Can design

be made scientific?", he answers that at least *some* features are beyond scientific attack. A closely related and even more critical question might be: "To what extent do we need to study the subjective thoughts and feelings of human beings — as distinct from what they *do* (which is how we study other organisms) — in order to understand

the history of processes such as the evolution of design?". And if the former seems important we may further ask: "How do we do it?" and then: "Can we do it scientifically?" □

Martin Pollock is Emeritus Professor of Biology, in the University of Edinburgh, UK.

New English Origin

Richard Dawkins

The Illustrated Origin of Species: An Abridged Edition of the Sixth Edition. Abridged and introduced by Richard E. Leakey. Pp.240. (Faber and Faber: London, 1979.) £8.95.

An invitation to review the *Origin of Species* in *Nature* is too much for the humble modern biologist to pass up. What line to take? "Would be better if he had stuck to pigeon breeding?" No, that's been had before. "Sexist, and unconsciously favours the status quo?" Better, but those in a position to see the funny side haven't the sense of humour to do so. "Cannot be saying anything very profound, since any layman can understand him?" Ah, but that does not mean that any *fool* can understand him. On the contrary he has been comprehensively, comically, misunderstood by many who were not laymen and not fools. The patient effort Darwin put into advocating his thesis, the persuasive, cogent clarity that he achieved, lead one to the salutary realisation that ideas do not have to be formidably complex, smothered in mathematical barbed wire, in order to be utterly incomprehensible to unprepared minds. They only have to be revolutionary. But this reviewer is getting above himself. It is his humbler task to review a pleasing picture book for the Christmas coffee-table market.

In a sense, everything is in the idea. Once you have *thought* of producing an illustrated *Origin of Species*, the rest is money for old rope. Well, and scissors and paste and a lot of hard work phoning round to the photographic agencies. I would certainly like to salute the unnamed employee (or ex-employee) of the Rainbird Publishing Group who thought of it. If ever an illustrated book conjured up vivid pictures in the mind of the reader, it is the *Origin of Species*. Darwin wrote before colour photography was invented, and he included only one technical diagram in his book. But he was such a passionate advocate, such a tireless seeker after honest ways to make his meaning plain to everyone, that I cannot doubt that he would have welcomed all the visual aid that

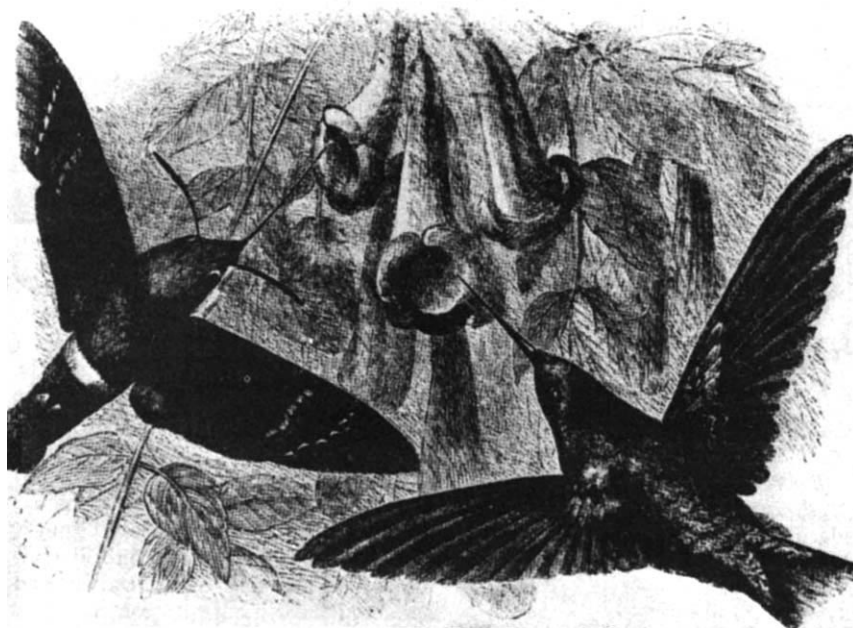
modern photography can offer.

The idea, then, is an excellent one, and I find nothing irreverent or reprehensible about putting it into practice, except insofar as it may pre-empt later bites at the same cherry. It is, evidently, an idea whose time has come, a hot property in the publishing world. Only about a year ago I recall a colleague, who had been invited by a publishing company to edit a similar illustrated *Origin*, discussing how he might do it. An artist as well as a scientist, he saw the task as a labour of love for Darwin, and he would have used only contemporary Victorian illustrations. I (who coincidentally and simultaneously had been invited to edit an illustrated *Origin of Species* by yet another publisher!) was more of the Leakey school of thought. If Darwin had wanted to illustrate his book with Victorian prints, I suggested, he could have done so himself. I would have used modern pictures, as technically perfect as I could find. I think Darwin would have appreciated that labour of love more, and I am glad that Leakey and the Rainbird team elected to do it that way. My colleague and I, incidentally, both declined our invitations, but I still have the notes I made as I reread the *Origin* with illustrations seriously in mind, and it was consequently of absorbing interest to me to see how Leakey and Rainbird had actually done it.

I think they have done it rather well. In addition to excellent black and white and colour photographs, they have used artistic drawings and paintings, clear maps and diagrams, and even a few Victorian prints. The captions are well done, frequently beginning with a relevant quotation from Darwin's text and ending with an up to date comment from Leakey. Someone who simply browsed through the pictures would come away with a good feeling for the diversity and complexity of life, with a sense of something needing to be explained which might inspire him to read the text.

I am less happy with the fact that Leakey has comprehensively bowdlerised the text into a kind of *Reader's Digest* version. No doubt some cuts were necessary; I am sure Leakey has succeeded in his stated aim of eliminating Darwin's "tendency to Victorian wordiness", and that he can be trusted to "in no way change Darwin's original meaning," but even so! This may be the only copy of the *Origin* that many people possess, and they will want to be able to refer to, or quote, exactly what the man said. It might be thought that the problem could have been solved by indicating omitted passages by dots, and

Humming-bird hawk-moth and humming bird, showing convergent evolution.

Taken from *The Naturalist on the Amazons*, by Henry Bates, and from the *Illustrated Origin*.

this would have been so if Leakey had contented himself with cutting out definite large chunks or whole chapters. But in fact he has done such a thorough (and actually rather good) editing job that scarcely a single sentence is exactly as Darwin wrote it, and the pages would have become a sea of dots. Leakey insists in his Introduction that, in making these cuts, he has not "tried to make Darwin more modern than he was, since he can stand on his own feet." But consider the very first whole sentence omitted: "There is, also, some probability in the view propounded by Andrew Knight, that this variability may be partly connected with excess of food." Darwin was wrong here, and the omission of the sentence (with no indication that anything has been cut) cannot fail to make Darwin seem more modern than he was. Darwin can stand on his own feet, certainly, and he doesn't need that kind of help.

Turning to the rest of the Introduction, I sympathise. The *Origin of Species* must be one of the most introduced books in science, and the supply of original things to say must be running low. In the circumstances Leakey does what I would have done, and contents himself with a smooth and unexceptionable record-sleeve job, a little standard history and a little modern biology, especially genetics, bringing us up to date on what has happened since Darwin's time. For me, as, I think, for Leakey, reflection on such matters has the odd effect of underlining Darwin's own remarkable prescience. He was, in Michael Ghiselin's words, "a genius of the first rank and a man of immense learning and he often worked a century or more ahead of his time." Natural selection theory is in an exciting and fast-moving phase just at the moment, but in many respects the 'revolutionary new' insights of the 1960s and '70s represent a rediscovery of something that had been lost — Darwin's own

uncompromising grasp of the ruthless individual struggle in the economy of nature.

For this reason I am slightly sorry that Leakey chose the sixth edition as the basis for his abridgement. The first edition, though not Darwin's parting shot on the subject, is of greater interest, both historic and scientific. It is of greater historic interest as the version that actually hit the Victorian solar plexus. But, paradoxically (this, too, is not an original point!), it seems scientifically more advanced than the sixth edition. The sixth edition includes Darwin's attempts to rebut contemporary objections to his thesis. These objections now have a fusty, dated air, and Darwin's replies to them inherit the same old-fashioned feel, something that the first edition, in its freshness, does not have. As is well known, Darwin's attempts to grapple with objections such as those of the great physicist Lord Kelvin, led him up the Lamarckian garden path much further in the sixth edition than in the first. Kelvin's calculation suggested that the Earth simply was not old enough to allow the evolutionary process that Darwin

postulated. Darwin, humble non-mathematician that he was, had to accept Kelvin's authority and this led him into erratic special pleading. As Eisely has remarked, "today there is a tendency in some quarters to regard the physical sciences as superior in reliability to those in which precise mathematical adeptness has not been achieved. Without wishing to challenge this point of view, it may still be worth a chastening thought that, in this long controversy extending well over half a century, the physicists made extended use of mathematical techniques and still were hopelessly and, it must be added, arrogantly wrong". Darwin had been right all along, in the first edition, and the objections to his theory that he anticipated and refuted in the first edition have proved the durable ones that we still meet. Leakey actually seems to have an impoverished view of the first edition: he says that Darwin, faced with the difficulties of natural selection, "began to search for additional mechanisms. Darwin wrote another book about one of these accessory mechanisms — sexual selection — and he discussed it briefly in the sixth edition of *The Origin of Species*." This seems, wrongly, to imply that the first edition lacks the section on sexual selection.

The illustrations in this book are well chosen and attractively presented. Even if they were not, it would be hard for the formula to fail. Open the *Origin of Species* anywhere, and you immediately feel you have walked into the presence of a timeless master, whose location in the Victorian era would be hard to guess but for his ignorance of Mendelism and his odd deployment of commas (which, now that I think about it, Leakey also seems to have taken it upon himself to correct). To complete my quotation from Ghiselin, "What a pleasure it is to bask in the sunlight of his intellect". The pictures don't spoil my pleasure, and if they help to spread some sunlight over the coffee-tables of Christmas I am all for them. □

Richard Dawkins is Lecturer in Zoology at the University of Oxford, and a Fellow of New College, Oxford, UK.

Highly popular science

THERE will be little in *Scientists at Work: The Creative Process of Scientific Research* (edited by John Noble Wilford; Dodd, Mead: New York; \$9.95), a collection of articles by journalists from the *New York Times*, that will be recognisable to working scientists. The articles are short, superficial, uncomfortably chatty and devoid of any critical approach to their subject. The writing is of the "Chilly winds howled across a massive stone overhang where a band of Stone Age

hunters huddled around a campfire" type. The actual steps of the tedious construction of the elements of Stone Age life are omitted in favour of flowery and simplistic imagery. And it is the same throughout this collection of the "highly popular" *Scientists at Work* series which "introduces a world rarely seen or imagined by non-scientists".

Why is science reporting so bad? The editor informs us that none of the nine authors have less than ten years reporting experience. In addition we find that no effort or expense was spared to get the stories. "Interviews . . . extended over a period of several days" and the work involved "staying up all night at Kitt Peak

Observatory . . . trekking through the California high desert" and even "flying low over a Minnesota lake on the trail of timber wolves". The full weight of "the largest and most experienced staff of science reporters of any newspaper in the country" has produced journalism that is poorly researched and badly written. Surely the US national newspaper of record can produce better.

A collection by Isaac Asimov (*Life and Time*; Doubleday: New York; \$9.95) is, in its own way, not much different. In place of nine reporters we have the Asimov factory — 198 books in 20 years. Asimov is a clear writer, now victimised by his own success. His *Fantasy and Science Fiction* essays are issued in book form "every 17 months", he writes for an amazing range of magazines from *TV Guide* to *Penthouse*; and he is on call to editors all over the country for articles on topics as diverse as special relativity, taxonomy, world government and space law. He is now a writer who tells his "Gentle Readers" that "my cheerful self-satisfaction is no evidence in itself that everything I write is routinely accepted". (An article on memory was rejected by *Playboy*.)

This collection, which includes cast-offs, previously unpublished works, out of print essays and duplicated material expresses little more than Asimov's "relentless determination to see that everything I have written (within reasonable limits) receives the relative permanence of book publication". Asimov has had little criticism of his work in his long career — and it shows. "I write as much as ever and the material I turn out seems to be as good as ever; not in my opinion only, but in those of my editors and readers who have no reason to lie to me".

Asimov has managed to avoid the errors of the daily journalists; he is thorough and generally accurate in reporting the facts. But his attitudes and opinions are superficial and anachronistic. There is a reflexive and unthinking defence of genetic engineering, a male chauvinist defence of the use of the word "man" in place of human, a superficial technocratic account of world politics and a nationally chauvinistic advocacy of space colonisation to be "carried through as wisely and as farsightedly as the colonisation of the American West". The Asimov of the negative solubility hoax has disappeared and he now writes as a pompous elder statesman out of touch with changing world conditions — from the job crisis in science to the emergence of revolutionary Third World political movements. His success is a success of the early 1960s and he shows little inclination to change his formula.

Joe Schwartz

Affection for birds

BIRDS give more pleasure to humans than any other class of creature, their own kind excepted. This pleasure takes many forms and finds expression in ways which not infrequently conflict. Many who delight in birds which visit their gardens deplore those who enjoy birds caged, who in turn despise the wildfowler, who deprecates the egg-collector, who dislikes the conservationist. Thus, each forgets that he is united to the other by a bond of common interest — affection, however expressed, for birds — and that sometimes one is in another's debt.

It is an intriguing irony that the conservation of Britain's most significant contribution to the world's avifauna is entirely dependent on a section of the shooting fraternity. The red grouse — a distinctive subspecies of the willow grouse — is found only in the British Isles and would be far less numerous than it is today were it not for shooting, while the moors where it lives would be sheepwalk or sitka spruce and biologically much less interesting. In *Going to the Moors* (John Murray: London; £11). Ronald Eden traces the social history of grouse-shooting and tells something of the development of moor management and shooting techniques. In the eighteenth century, the shooting of upland game was a rugged and expensive affair — a rich man's expedition which, though the support staff might bring up supplies of cheese, sherbets and champagne, was nonetheless a serious operation. Then the romantic poets began to work their influence on good taste, which had long eschewed wild landscapes; Albert and Victoria set the seal of approval on the Highlands and suddenly the numbers of those travelling north became a multitude. In response, traders and entrepreneurs were quick to smooth the way and line their pockets. By the latter half of the nineteenth century the grouse season was part of the social calendar. Grouse management slowly became a science, designed to produce the maximum

number of birds for the guns. But the grouse ceased to be a bird, a thing of beauty with a biology and behaviour of considerable intrinsic interest: for most of those involved it became a symbol and an excuse for quite unrelated pleasures: even those who actually saw and shot the birds were preoccupied with bags and performance. The book is copiously illustrated in black and white and entertainingly written to appeal to those who are or would like to be part of the grouse season society.

It is a far cry from the organised and luxurious pursuit of grouse to the unpredictable and often uncomfortable pursuit of greenshank. Desmond Nethersole-Thompson's dedicated and untiring study of this uncommon and elusive species began in 1932. With his wife, he describes in *Greenshanks* (T. & A.D. Poyser: Berkhamsted, UK; £8.80) not only their findings on the biology and ecology of the birds but also the trials and rewards that have been part of the experience. Greenshanks breed in the most lonely and desolate parts of Britain — places like the flows of Sutherland which not a few naturalists dismiss as "Mamba" — miles and miles of nothing at all. But to the Nethersole-Thompsons it is clearly a place of enduring fascination. The wildness and the wetness of this still almost immaculate wilderness are for them not the least of its charms and one must admire the cheerful acceptance of storm-bound days huddled in sleeping bags, thus saving food and fuel, until the weather improves enough to permit a return to field work. Greenshank nests are notoriously difficult to find and were once the "Cordon Bleu" of the egger: in describing the problems of nest-finding, the book conveys much of the author's own enthusiasm, and throughout there is a pleasant and rare mix of thorough scientific analysis with abiding affection for the subject. Though centred on the authors' own findings in Scotland, the book draws together other sources in Britain and Europe to form a comprehensive appraisal of current knowledge. Occasionally, the casual reader



Walking-up Grouse, by Edward Neale, 1893

Taken from *Going to the Moors*

Joe Schwartz is on leave from the City University of New York.

will wish to skip minutiae but it is no disadvantage that they are included for the specialist.

Rarity and the challenge it represents appeal to many naturalists and thus they may overlook opportunities for research and enjoyment on their own doorsteps. The town pigeon lives a life of dependence on man, treating his buildings like the cliffs inhabited by its wild progenitor the rock dove and treating man himself like the cattle or goats amongst which rock doves walk unconcerned. In *The Public Life of the Street Pigeon* (Hutchinson: London; £6.95), Eric Simms takes a comprehensive look at a bird which has lived with man for well over 6,000 years. The first part of his book looks at the general biology and relationships of the order, and at the pigeon's past and present relationships with man, who pets it, eats it, races it, breeds it into bizarre forms, uses it in war and wages urban war against it, experiments with it or on it, and is photographed with it standing on his head. While grouse shooting has the social cachet and is today a not insignificant earner of foreign currency, pigeon racing outstrips it on several counts with, at Simms' estimate, several million birds kept by 100,000 pigeon fanciers and exceptional individual birds changing hands for several thousand pounds. One thing might unite these interests — a common dislike of that impartial predator the peregrine falcon. In

fact, it was the pigeon racer's outcry that encouraged British conservation bodies to look at peregrine populations 20 years ago and thus discover that they were declining because of an unrecognised group of environmental pollutants, the organochlorines. The second half of the book is devoted to the street pigeon at home, its biology and behaviour, revealing how much of value and importance can be learned from the commonplace. The book contains much information, presented in succinct and entertaining style and generously illustrated in black and white.

Man's relationship with parrots is also one of some antiquity but founded on different emotions. Pigeons lack the anatomy to swagger or swear. Parrots do not provide us with a source of fresh meat in winter. The modern pigeon fancier is much preoccupied with successful breeding but the parrot keeper apparently sees his bird more as an ornament than an organism, making little attempt to provide conditions under which the birds can behave reasonably naturally, let alone breed. *Lovebirds and Related Parrots* by G.A. Smith (Paul Elek: London; £15) necessarily reflects this attitude as well as reminding us of how little is known about birds which do not occur in Europe or North America. Thus, the section on ethology occupies four pages compared with that on "simple genetics, colour mutations and colour definition" which runs to thirteen, plus an apology for compressing the subject into so small a compass. The bulk of the text summarises the pitifully little that is known of individual species in the wild. To give fair credit, the author places great emphasis on captive breeding — aviculture can no longer regard wild populations as an inexhaustible source of supply: its own depredations have had severe effects on rarer species and many others have suffered greatly from recent habitat changes. By drawing attention to problems and partial solution, the book could be a valuable contribution to the conservation of parrots.

Though scientific ornithology has only lately begun to discover much of birds' basic biology and behaviour, they were not unnoticed by previous generations, whose dialect names can provide useful insight into the earlier distribution and abundance of species. *All the Birds of the Air* by Francesca Greenoak (André Deutsch: London; £6.95) collates many, but not all, the British names. Some one suspects of being Victorian contrivances and there are some mis-attributions. The author gives a very personal view of each bird's place in lore and literature, making a pleasant if sometimes inaccurate read for the sentimental bird lover.

Christopher Perrins combines accuracy with readability in *British Tits* (Collins: London; £6.50). In the best New Naturalist tradition, the author covers the seven species of British tit, drawing



Coal tit (*P. ater*)

together all available material from sources throughout their world range but in particular from Holland, where studies have gone on almost without a break since 1912, constituting one of the largest series existing for any bird anywhere in the world. In Britain, intensive study at a site near Oxford began in 1947 and has continued since, providing not only a direct opportunity for observation but also a comparison between the British and Continental subspecies living in subtly different woodlands. Tits are apparently a highly intelligent order, having mental and manipulative skills evolved for the survival of invertebrate eaters in a cold climate. As such, they are of particular interest in behavioural studies. The book commences with a chapter summarising the biology of each species and then goes on to examine them as a group, comparing ecological differences, food requirement, feeding habits, breeding, population dynamics and the relationship between tits, their prey and predators, respectively. The illustrations are apposite and there is an exhaustive bibliography and index. Perrins' style is eminently readable: a mass of complex information is organised with an unobtrusive precision that belies the meticulous care necessary in such work. Nor is he afraid to develop into the field of stimulating speculation an argument based on observed fact. There are still all too few such studies.

John Andrews

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, UK.

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Birdwatching guides

NEW bird books keep coming on the market at such a rate that it is difficult to keep up with them or to know which ones are worth having. This review covers six smallish books which have been published in the past twelve months or so, mostly very recently. As will be seen, the books are quite disparate in their aims, so much so that comparisons between most of them are not profitable: the choice will be governed by the reader's requirements.

A Field Guide to the Seabirds of Britain and the World by G. Tuck and H. Heinzel (Collins: London; £5.25) is the most specialised of the works discussed here. This is a "standard" type of field guide, with a coloured picture, often showing more than one age group or plumage, of each species together with a descriptive text and a distribution map. The text concentrates almost entirely on identification/plumage and range. However, short sections at the beginning of each family or group amplify this with descriptions of behaviour and nesting habits. The text also contains a large number of line drawings, some of them adding additional identification features.

As already mentioned, this is a book for the *afficionado* or the globe-trotter. Although it covers almost 300 species of seabird, only a couple of dozen of them breed in the United Kingdom and several of these are rare. This edition has an extra 24-page section at the back describing British birds in greater detail with larger distribution maps for the British Isles only; apparently there will be later editions in which the resident birds of other areas of the world will be covered in greater detail.

It is a difficult task to attempt but, although it is not without its faults, it has on the whole succeeded. Both plates and text will be valuable for bird-watchers; and in ecological terms it certainly fills an empty niche. My main comment to the user would be a word of caution. The text describes differences (for example, between the shearwaters) giving the impression that the species concerned can be reliably separated in the field. Some of the characters given, such as leg colour, can be virtually impossible to see unless the bird is in the hand. In areas where numbers of closely related species occur, it takes a very experienced fieldworker to identify the birds with any degree of reliability.

Wetlands — estuaries, marshes or even reservoirs — are often rich in birds. Malcolm Ogilvie's book *The Birdwatcher's Guide to the Wetlands of Britain* (Batsford: London; £4.50) describes the main wetlands of England, Wales or Scotland (but not Northern Ireland), how to get there and what one is likely to see there. The text concentrates on the better areas in each county and on the wildfowl

and waders. These two groups of birds are often present in impressive, even spectacular numbers, and in recent years, because pressures on land use have led to many 'development' plans for such 'waste' ground, ornithologists have been counting the birds on these areas. Doubtless partly because of our relatively mild winters, they have found that our wetlands harbour enormous numbers of wildfowl and waders. Birds from other areas of Europe and from the Arctic visit us in great numbers. In mid-winter British wetlands may play host to very large proportions of the populations of these birds; and therefore the conservation of our wetlands is of pressing urgency.

The book is divided by county and each major water is briefly described with information on how it might be reached. There follows a list of the most important species of wildfowl and waders together with the range of numbers which may be expected. Although more detailed figures are available to the specialist, for the casual reader these are the only figures readily available. Other species of special interest in each area are mentioned very briefly; I would have welcomed a slight expansion of this section. The book will be useful, especially to those who like to travel some distance for their birdwatching.

Peter Conder's *RSBP Guide to Birdwatching* (Hamlyn: London and New York; £2.50) is a useful little book and good value for its price. I found the title not wholly descriptive of the work. The early part is indeed a useful initiation to birdwatching, covering books, binoculars, places to go, how to keep records, and so on. However, almost two-thirds of the book comes closer, to my mind, to bird study. It covers aspects such as counting birds (usually not as easy as it may seem at first sight!), the lives of birds (flight, feathers, moult, bill, legs, and so on), migration and ringing, territory and song, nesting, distribution, habitats, ecology and food. In each of these sections the subject matter is described simply and clearly, and the ways in which the reader might make his or her own observations are suggested. There is a final section on conservation and protection, and there are useful appendices on books and other literature of ornithological societies. Useful for the beginner.

The Birdhouse Book by D. McNeil (Pacific Search Press: Seattle, Washington; paperback \$8.95) contains useful sets of instructions on how to build nest-boxes and bird-feeders of a wide variety of designs. Beware, however, if you fancy the design of a nestbox, that the bird for which it is designed breeds in Britain, as the book was written for the American market. Although this is a well-illustrated book with clear construction plans, a better work for European readers is the booklet *Nestboxes* published by the British Trust for Ornithology (Tring, Herts, UK).

Discover Birds by Ian Wallace

Alzheimer's Disease

Early recognition of potentially reversible deficits

Edited by A. I. M. Glen and L. J. Whalley

1979 228 pages illustrated
hardback £14.00

Organ Preservation II

Edited by David E. Pegg and Ib A. Jacobsen

1979 344 pages illustrated
Hardback £21.00

Cimetidine

The Westminster Hospital Symposium

Edited by Christopher Wastell and Peter Lance

1979 324 pages hardback
£15.00

Functional Histology

A text and colour atlas

P. R. Wheater, H. G. Burkitt and V. G. Daniels

1979 288 pages 387 colour, 83 black and white and 69 line illustrations
hardback £19.50/paperback £12.95

Laboratory Methods in Antimicrobial Chemotherapy

Edited by D. S. Reeves, Ian Phillips, J. D. Williams and R. Wise

1978 288 pages illustrated
hardback £17.00

Scheduled for MARCH publication

Histochemistry

Theoretical and Applied
Volume I Fourth edition
Preparative and Optical Technology

A. G. Everson Pearse

Robert Stevenson House,
1-3 Baxter's Place, Leith Walk,
Edinburgh EH1 3AF

Churchill

Livingstone 

(Whizzard Press/André Deutsch: London; £4.95) is, as its name suggests, an introduction to bird-watching. It introduces the subject with sections on note-taking, how to identify birds, and so on. It then goes on to take the reader to seven selected bird-watching areas, such as the Isles of Scilly and the Cairngorms. For each, it describes the area fully and then the kinds of birds that one might see there. It is an attractive narrative without going into too much depth. The aim is to describe the typical birds of different areas rather than all the birds in Britain. It is lavishly illustrated by the author's pleasing line drawings and coloured sketches. In addition there are many photographs by Philip Sayer; the black-and-white ones do not always seem to have reproduced well, but the coloured photographs of the habitats are, as a group, among the best I have seen anywhere.

Alan Richards' *British Birds: A Field Guide* (David and Charles: Newton Abbot, UK; £4.95) is a departure from the standard field guide which illustrates each species with small coloured sketches designed to show the key field characters. Many current field-guides include the birds from a very large area; the British beginner

must therefore sort through large numbers of species which are unlikely to occur. This book concentrates on British breeding species plus the regular winter visitors. On the whole the selection seems pretty good, though a few birds likely to be seen by the beginner get only a brief mention (for example, Bar-tailed Godwit, Great Skua, Firecrest, Rock Pipit). In this book there are two illustrations for each species, a line drawing and a coloured photograph. The text amplifies the pictures for the description and carries extra information on voice, habitat, nest and status. On the whole, this is a successful work and will be a useful addition to the beginner's library. The photographs are usually adequate for identification, but I wish fewer of them were taken at the nest, as this does not always give the right 'jizz'. For example, the waders are usually seen in other habitats where they look quite different. Also, at times, the bill shape is obscured by a beakful of insects. Apart from this, they are a pleasing collection of photographs and the general production is attractive.

C.M. Perrins

C.M. Perrins is Director of the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

Pretty birdies

The series of books of paintings and photographs reviewed here reflects again the publishers' interest in the market provided by the ever-growing number of those attracted by birds: in the last four years over 100,000 people have joined the RSPB, bringing its total membership to over 300,000. Sadly, the contents of books published for this market are often of poor quality: either the paintings or photographs are hardly worth publishing — or buying — or else the text is ordinary, repeating *ad nauseam* the description of birds and their behaviour that can be found in the usual textbooks. Sad it is, too, that few authors whose books are reviewed here have tried to match the artist or photographer with original or more personal observations.

An example of this discrepancy is illustrated by the *Country Life Book of Birds of Prey* (Country Life Books/Hamlyn: London; £20). Gareth Parry, a young Welsh artist still in his twenties, is a meticulous draughtsman who paints almost every barbule and barbicel. But he has not mastered the art of perspective and some birds look flat: a young kestrel really has got its underparts in a twist. Parry also falls into the habit of portraying breaks in the feather vanes, presumably to give a verisimilitude to the picture which is in fact artificial. In spite of these criticisms, however, Parry is a promising artist of the barbicel school of painting. Photographic as his pictures often are — and I am not implying that they are copied — they capture the atmosphere of the bird and its habitat; the winter paintings of the gyrfalcon on an estuary and the white-tailed sea-eagle made me shiver. The book covers all British diurnal and nocturnal birds of prey, but the vignettes by other artists seem out of place. The text by Rory Putnam is competent but it contains nothing original. The book is worth looking at but at £20 I wonder if we are not paying for the name of the publisher.

T. Lambert and A. Mitchell's *Birds of Shore and Estuary* (Collins: London; £6.95) is another coffee-table book produced for popular appeal, with just over 50 paintings of a range of sea and shore birds. Like Gareth Parry he paints in great detail but not so accurately. The colouring of the feathers, as well as the shapes of some of the birds, is so unreal as to make identification a puzzle, and it is difficult to see how some of the birds depicted flying could get their wings into the positions the artist has contrived for them. Alan Mitchell writes a short essay introducing the four groups of paintings: gulls, terns and skuas; breeding birds; ducks and geese; and finally, birds of estuaries. Each picture is accompanied by a short, popular commentary of about 300 words.

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A contrast to these books is found in Réna Fennessy and Leslie Brown's *Birds of the African Waterside* (Collins: London; £12), although it is another large book of 24 paintings depicting 30 species. The paintings are warm, as befits a book on African birds. Her painting is less representational than that of Parry and Lambert and is generally very successful, although the white-starred bush robin has a badly tailored skin with wrinkles down its back — another painter's cliché which may fool the unwary. The remaining paintings are attractive studies of the waterbirds most likely to be seen on the edges of African lakes and rivers. The text is further decorated with sketches of birds and habitats. Sadly, two plates are transposed: those of the collared pratincole and the three-banded plover.

As one would expect from Leslie Brown, the text has original touches: the introduction is an account of the author's safaris up and down rivers and lakes rather than an essay on the different types of wetland, although as Leslie Brown has wandered over great sections of Africa he arrives at a good summary anyway. The text accompanying each of the plates contains more original observations and anecdotes than one usually finds in a book of this sort.

The late C.F. Tunnicliffe's *Sketchbook of Birds* (Gollancz: London; £7.95), introduced briefly by Ian Niall, is by far the most worthwhile of the books of paintings I have been reviewing. It is made up of 132 colour reproductions of pages from his early sketchbooks dating from the early 1930's to the early 1950's. The sketches are arranged in five groups: sea birds; waders; geese, ducks and swans; birds of prey; and finally miscellaneous birds. They are of value to the serious ornithologist and bird artist and will give lasting enjoyment to all who, in one way or another, are intrigued by birds. Often detailed, the feather by feather drawings, from which the later paintings were made, show changes in plumage from season to season, different feeding methods, a range of typical postures — relaxed, wing stretching and so on. Others are studies for future pictures in water colour or in crayon. On some of the plates Tunnicliffe's own descriptive notes appear. Legends give details of identification, place and date of drawing. These sketches confirm him as an outstanding artist, arguably one of the greatest bird painters of all time, ranking with or above Bewick, Audubon, Liliefors. Since the present group of sketches was taken from the earliest sketch books I would hope to see more of his later sketches. Finally, as well as being the best book in this group it is also the least expensive.

Eric Hosking's Birds (Pelham: London; £10.50; published as *A Passion for Birds* by Coward, McCann and Geoghegan: New York; \$25) is an excellent title for a selection of photographs by the grand

master of his profession. What Tunnicliffe was to bird painting Eric Hosking is to bird photography and, like Tunnicliffe, his photographs have stimulated the growth of interest in birds. The book consists of some 200 photographs, many of them classics, of which 80 are in full colour. It is often the reproduction of the photographs which has let this book down: some of the photographs have been cropped and enlarged to the extent that definition is poor and the rendering of the colour photographs rather pale. Eric Hosking and Kevin MacDonnell have contributed a short text in 13 chapters, a mixture of rather elementary nature notes and interesting photographic information. It is a pity that Eric Hosking did not write more about his photographic experiences. Finally, full photographic details of each photograph are given in the appendix. In spite of these criticisms it is still a very worthwhile book, with many photographs which are not only beautiful to look at but from which it is also possible to learn.

The bird photographs of Frederick Kent Truslow, a photographer well known in the US, have been treated rather differently. In *The Nesting Season* (Studio Books/Viking: New York; \$25; Elm Tree Books/Hamish Hamilton: London; £15), the photographs are arranged in the sequence of the breeding cycle. The first half of the book, which is dominated by a popular but well written account of the nesting season by Helen D. Cruickshank, is illustrated by black and white photographs, of which few occupy more than a quarter of the page, thus giving the text more prominence than is usual in a book of this kind. The photographs lose nothing; they are all pin-sharp and seem much better printed than in *Eric Hosking's Birds*.

The second half of the 136-page book is entirely composed of colour plates, most of which are excellent but others have been over-enlarged, depicting the bird at least twice life-size, resulting a poor definition and colour reproduction. In those where the picture is given a two-page spread the middle portion of the bird is lost in the binding. Some photographs are superb, particularly the blue heron, mutual preening of moorhens unfortunately labelled "battle for dominance", and the American national bird — the bald eagle — has a striking portrait. Unique are three photographs showing a pileated woodpecker carrying its three eggs to a new site after the top of the nest tree had broken off, exposing the nest. An appendix gives some of the photographic data but lacks details of film stock and camera.

The five volumes published by Orbis entitled *Birds of Ocean and Estuary*, *Birds of Mountain and Moorland*, *Birds of Marsh and Shore*, *Birds of Heath and Woodland* and *Birds of Hedgerow and Garden* (Orbis: London; £7.50 each) have made two previous appearances in Europe as a part-work, first in Italy and then in Britain under the title *The Orbis*

Encyclopaedia of Birds of Britain and Europe. In Italy it was nothing more than an encyclopaedia of European birds, describing 642 species in systematic order with poor paintings of birds — nearly all the beaks, for instance, were too small, the colours were not always representative and often the shapes are inaccurate. For the English part-work John Gooders made the best of a bad job and wrote a number of popular essays on various aspects of a bird's life, added many excellent photographs and, particularly useful, colour plates of the wing patterns of nearly every group of birds. This third version is the same English part-work now in five hard-backed volumes with the misleading titles given above. The titles are misleading because as the cover admits in small print, the work lists the birds in systematic order. Consequently, the first volume, entitled *Birds of Ocean and Shore*, includes most ocean or shore birds but omits waders, passerines and so on which regularly visit the shore. The same criticism can be made of the other volumes. *Caveat emptor* applies and the £37.50 for the five volumes might be better spent elsewhere.

Peter Conder

Peter Conder is a writer and a wildlife management consultant who has recently been working for IUCN/WWF in Pakistan and Jordan.

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Invertebrate Neuropharmacology

Lucy D Leake
Robert J Walker

The invertebrates, with their less complicated nervous systems, are being increasingly used to model the neuronal activity of vertebrates. In reviewing progress in the field to date, the authors of this book highlight the benefits and shortcomings of the applications of invertebrate studies to the vertebrates, and indicate possible future developments. A comprehensive, current bibliography is an important feature of the book.

This will prove an important source book in a developing area for researchers in pharmacology.

North American Rights: John Wiley (Halsted Press)

384pp 85 illustrations
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Wildlife in danger

THE conservation band-wagon trundles on, dropping a seemingly endless shower of new books about endangered species and their approaching extinction. One may wonder how many hundred square miles of virgin forest are clear-felled every year to make pulp for the paper on which they are printed, and what happens to the inhabitants, plant and animal, of the biome destroyed. Some of the books are worth the sacrifice, but as for others — less talk might result in more conservation though fewer royalties for the riders on the wagon.

Australian Endangered Species (Cassell: London; £15) by Professor Derrick Ovington, formerly Professor of Forestry at Canberra and now Director of the Australian National Parks and Wildlife Service, is one of the best books on the problems of the conservation of nature to have appeared for a long time. Although it deals specifically with the fauna of Australia, much of its information is applicable to any part of the world. The author writes as a scientist and deals with his subject objectively without sensationalism, and the resulting clear and sympathetic account of the history, present state and future prospects of the Continent's fauna and flora makes fascinating reading. The early white pioneers, isolated in an alien world and facing incredible hardships, understandably felt an overwhelming urge to subdue and transform the wilderness in order to survive. Many species of the fauna were abundant and were killed for food or recreation, or, when farming was established, as vermin. The early settlers did not understand that breaking-in the wilderness for agriculture or stock-raising so altered the environment that some native species could not survive. Furthermore they could not foresee the dire results of the well-meant introduction of alien species for practical or sentimental reasons. The extinction or extreme rarity of many species is attributed in frequent instances to habitat alteration by stock or rabbits, and to introduced predators such as the cat and the fox, so that species once widespread and abundant have suddenly become scarce or disappeared. "Like fresh water or clean air, wildlife is liable to be taken for granted and its value underestimated until supplies run short".

The heart of the book deals with the 23 Australian mammal, 18 bird, and 2 reptile species that are regarded as endangered. Each species is depicted in a full-page coloured illustration facing a page with its description and biological details, and a map of former and present distribution; the pictures are beautifully done and well printed. The book concludes with an account of what has been done, and what remains to be done, in conserving Australian wildlife, emphasising the need

for massive further research and for education: "the dissemination of information about wildlife to many people and agencies is essential if sympathy with wildlife protection measures is to be translated into active support and adequate funds are to be made available." This is a splendid book that will be valued by specialist and layman alike.

The Living Swamp (Orbis: London; £5.95), by A. Borgioli and G. Cappelli, describes the different kinds of marshes, swamps, fens and bogs that occur throughout the world, and illustrates their flora and fauna with many excellent colour photographs, all of them beautiful and many dramatic. The first of the four chapters deals with the aquatic environment and explains the origin and evolution of marshland, pointing out its inherent instability under the influence of natural forces as well as under the activities of man. Many are threatened with destruction through efforts to reclaim wastelands for agriculture or other purposes, such as the construction of airports for commerce or marinas for amusement. The second chapter displays the "marsh microcosm" and the food chain, ranging upwards from unicellular algae to vertebrate animals, based on the rich primary production of organic material synthesised by plants. The illustrations of this chapter include many unusual photographs of invertebrates, mainly insects, and excellent pictures of amphibians and reptiles. A well illustrated chapter on birds, "the lords of the marsh", then follows, pointing out how few mammal species compared with the host of birds are able to take advantage of this environment. The final chapter describes the characteristics of the major marshlands of the world and lists them by countries, noting the national parks and reserves where conservation of this specialised habitat is practised. The text well matches the illustrations to make the volume more than merely a coffee-table book; it has, however, suffered here and there in translation from the Italian, particularly

with names of species, as where it mentions "greytag" geese in Argentina.

In 1903 certain sportsmen, fearing that the stocks of big game that they hunted were diminishing, founded a Society for the Preservation of the Wild Fauna of the Empire, with the object of establishing game reserves and preventing the natives poaching the animals the alien whites wished to kill; no wonder the popular press lampooned them as "penitent butchers". Mr Richard Fitter has adopted the nickname as the title for his short history of the society (*The Penitent Butchers: 75 Years of Wildlife Conservation*; Collins/Fauna Preservation Society); Sir Peter Scott has done the drawings. Wildlife protection has always begun as an attempt to preserve game animals from overshooting or overhunting: "one of the few amenities that could be offered to soldiers (officers only, of course) and administrators marooned in darkest Africa . . . was shooting big game." The move from the protection of game to the protection of all wildlife came slowly, and it was not until well after 1945 that the conservation of whole ecosystems gained acceptance. The author devotes a chapter to breeding rare animals in captivity in order to return stocks to the wild, a measure that has had several successes, but is a last desperate remedy that for many reasons cannot hope to be repeated often.

The difficulties of breeding rare animals in captivity to save them from extinction are discussed in some detail by Sheldon Campbell in *Lifeboats to Ararat* (Weidenfeld and Nicolson: London; £6.50). This book, by a former zoo-keeper, is a chatty anecdotal account of life "behind the scenes" in zoos round the world and particularly in the San Diego zoo of California. The author says, "no activity by zoos has received more lip service and been less seriously undertaken than captive breeding. All zoos subscribe to the idea, but few are really doing much about it." A major obstacle to success in returning captive-bred animals to the wild is the difficulty of getting them to earn their living when they have known only the soft life of a zoo "welfare state". The difficulties are such that the author concludes: "the probability is that zoos will hold many captive-bred species far into the foreseeable future, and, possibly, some for ever."

The truth of this statement is well illustrated by Peter H. Beard in *The End of the Game: The Last Word from Paradise* (Collins: London; £10), an unusual but interesting and instructive book. It is the story of the elephants of the Tsavo region of Kenya, teeming with wild animals at the beginning of the century, but which, after being made a National Park, degenerated into an empty desert strewn with dead trees and the skeletons of the elephants it was intended to preserve. When the wardens and rangers of Voi Force finally stamped out poaching in the Tsavo Park in the 1950s they unwittingly sealed the fate of Tsavo,



Taken from *Lifeboats to Ararat*

Karen Blixen and Ingrid Lindstrom, Ngong, 1921

Taken from *The End of the Game*

for the elephants multiplied, and when the great drought came they destroyed their habitat and perished in the wilderness they had made. The book is lavishly illustrated with photographs of animals and men from the early days of colonial settlement until the present, a fascinating collection. The coming of the white man who built a railway and imposed his will on the land and its inhabitants was "attended by glory and courage, ennobled by sacrifice, enriched by science, medicine and law. But it marked

the beginning of the end in a land where nature herself had always been sovereign . . . It is too late to undo what has been done . . . we have conquered nothing at all."

L. Harrison Matthews

L. Harrison Matthews was formerly Scientific Director of the Zoological Society of London, and a member of the scientific staff of the Discovery Expedition, 1924-28. He has also done zoological research in the Arctic, South America, and Africa.

Making a living

THE word 'predator' immediately conjures up visions of a lion tearing at a carcass or of a peregrine falcon swooping on to its prey, but smaller and more numerous predators are at work everywhere if only we look for them. There is all the drama of the predator and its prey when a wasp butchers a butterfly or when a dragonfly pursues its insect victim over a garden pond. *The Hunters* (Hamlyn: London and New York; £5.95) by Philip Whitfield takes an imaginative and exciting new look at the world of predators and will inspire student and layman alike. It covers the whole range of hunters from tiny single-celled protozoa to large mammalian species. There is an introduction on population numbers and food chains and then a series of chapters that

group predators together under the methods they use for hunting.

Tigers, praying mantis and chameleons all hunt by stealth, relying on concealment to enable them to get close to their quarry. Some animals hunt in groups like soldier ants, pelicans and hunting dogs. Others, such as burying beetles and vultures, scavenge for dead animals. Ciliate protozoa, flamingos and baleen whales all use filtering mechanisms to catch their aquatic prey. There are also chapters on predators that use tools or traps and those that are specialised in the food they eat or in their methods of detecting prey. Every predator is given a double-page spread of text and diagrams which explain its hunting method; and the book is illustrated with some superb drawings by Richard Orr and Michael Woods. These include a walrus disembowelling a narwhal, an anemone engulfing a fish, a boa constrictor crushing an ocelot and a brown bear sweeping a

salmon out of a torrent.

These two artists have produced some more beautiful and informative diagrams and drawings for another book in the same series, *The Animal Family* (Hamlyn: London and New York; £5.95) edited by Philip Whitfield. This looks at the diverse ways in which animals find mates and rear their young. Like the previous book, it illustrates various topics with reference to examples chosen from the whole Animal Kingdom. The mechanics of sexual reproduction are explained, including the structure and function of the reproductive organs, hormones, fertilisation, and the strange animals that change sex. There are clear diagrams illustrating the variety of visual and vocal courtship displays, territorial behaviour and nest sites. Some examples of family life are considered in detail, such as the caste system of social insects, the complex social relationships in monkeys and apes, and parental care in crocodiles.

It is a pity that the text suffers so badly from the fact that several authors have contributed to the book. In one chapter the selfishness of courtship is rightly emphasised together with the conflict of interests between male and females and the diverse ways in which males compete with each other for the opportunity to mate. In the next chapter, however, we read all about harmonious pair bonds and are told that sneaky male mating strategies are not favoured because this would be harmful to the population. We even go right back to the old, and surely wrong, view that territorial behaviour exists to ensure that the population never grows too large and that fighting is ritualised for the good of the species.

The migration of animals has astonished man since the earliest times. The first explanation of the seasonal emigration of some species and the simultaneous appearance of others was the transmutation hypothesis. In summer, robins were supposed to change into restarts and hawks into cuckoos. Swallows were imagined to sleep all winter in the mud at the bottom of ponds while geese were thought to grow from barnacles. We now laugh at these old ideas but in *Animal Migration* (Orbis: London; £5.95) John Cloudsley-Thompson shows that the true explanations can be just as strange.

Tiny warblers flicker by night across thousands of miles of desert and ocean; spiders balloon about the skies on threads of silk; and salmon return from the sea to the exact streams in which they were born. There is a chapter on how the Sun, the stars and the Earth's magnetic field are used for navigation; and then the bulk of the book is divided into three sections: migration by air, land and water. Examples from all over the world are described with a clear and lively text, illustrated by abundant drawings, maps and colour photographs. All extremes are considered from the few centimetres that limpets wander round their

Taken from *Animal Days*

home base on a rock to the vast wanderings of the shearwater over the polar seas.

There are good accounts of the migration of large mammals, the movements of plankton, fish, turtles and whales in the sea, and of bird, bat and butterfly migration in the air. It is emphasised that animal movements on such a vast scale, without respect for international frontiers, present serious problems for conservation. What is the use of restricting whale kills in one part of their range if the restrictions are violated when they migrate elsewhere? The book is an excellent portrayal of the wonders of migration but has little to say on its evolution or adaptive significance which is dismissed as "one of science's many unsolved mysteries". Migration is said to be a widespread "instinct" of advantage to the species, which obscures the important point that different individuals within a species may migrate in different ways. Readers are referred to Robin Baker's recent book, *The Evolutionary Ecology of Animal Migration* (Hodder and Stoughton: London, 1979) for an up to date account of this problem.

In *Colour for Survival* (Orbis: London; £6.95) Peter Ward looks at the ways in which colour influences animal behaviour and ecology. The book is illustrated with spectacular colour photographs most of which are by the author himself, who has travelled widely in Africa, Asia and Central America and has first-hand knowledge of the animals he describes. It gives us an exciting insight into the use of colour in animals for camouflage, mimicry, warning and display. There are some marvellous examples, such as the caterpillar that looks like the seed-packed excrement of a frugivorous bird and another that resembles a feather stuck to a wet leaf. The diverse uses of colour are described in insects, lizards, frogs, birds and large mammals. My favourite is the flower mantis from Borneo which has bright white petal-like extension of the body that lure flower visiting insects to their unsuspecting death.

Animals and their World (Blandford: Poole, UK; £8.95) by Mary Parker Buckles, which is subtitled "The astonishing story of how mammals live in

their own ecological zones", left me mystified rather than astonished. The book is divided into chapters which deal with the major habitat types such as tropical and temperate forests, grasslands, tundra, desert, and the oceans. Every chapter begins with a brief ecological description and there then follows an idiosyncratic list of some of the mammals of the habitat with photographs and brief notes on each species. There is little relationship between the examples given and the ecological comments at the start of the chapter, and no attempt has been made to show how the different species are adapted to their various habitats. Because the book restricts itself entirely to mammals, there is no detailed account of how plants and other animal types influence mammalian lifestyles and the reader is left in a sea of examples wondering what to conclude.

A second book which uses the same plan, but with great success, is *The Living World of Animals*, another outstanding publication by the Readers Digest Association (London; £9.95) in association with the World Wildlife Fund. The main part of the book is devoted to sections on the different ecological zones of the World and shows, with an authoritative text and an abundance of excellent colour photographs and diagrams, how animals make a living in their various environments. There are also chapters on animal distribution, evolution and behaviour, and relationships with man. The book manages to convey the beauty and magic of the natural world, from the cold white silence of the polar regions to the richness of the tropical forests, and at the same time gives an up-to-date account of our knowledge on animal adaptations. As such it must be one of the best natural history bargains on the market.

Desmond Morris has spent most of his life surrounded by animals, from student days with a laboratory that was filled with dozens of fish and birds to the years as curator of mammals at London Zoo where his office was a cage with a desk inside! In *Animal Days* (Jonathan Cape: London; £5.95) he recalls the people and events that have influenced his life. The result is a book that is great fun to read, a mixture of rich observations on animals and stories that

are always amusing and sometimes hilarious. With varied interests that included film-making and paintings as well as Natural History, it was lectures by Niko Tinbergen and Konrad Lorenz that finally inspired Morris to take up zoological research. Baptised and then confirmed by these two great fathers of ethology, he moved to Oxford to study for a doctorate under Tinbergen. Here he made some fascinating observations on homosexual behaviour in sticklebacks and divorce in finches. He reared a pet crow that invaded nearby gardens and learned to squawk "Go away" in the cultured accent of an Oxford don. There were amusing meetings with J.B.S. Haldane and Julian Huxley and a wonderful story about Lorenz and a tame raven which I will not spoil for the reader.

Later on, in London, the author started the successful weekly television programme 'Zootime'. In front of the cameras the animals did not always behave as expected, like the time that the giant tortoise, roused to great activity by the warmth of the television lights, charged around knocking over the cameras and crew. The days at the Zoo also showed how sometimes hand-reared, captive animals could be reduced to hopeless mental hybrids, like the panda Chi-chi who preferred humans to her Russian spouse An-an. The famous artistic chimpanzee Congo and Guy the gorilla also rejected their own species as mates. Ethological research has helped us to understand the social and environmental needs of different species and Morris emphasises that modern zoos must provide these so that we can see the animals behave in their normal way.

There is, however, no substitute for observations in the field, although this can often be a time-consuming and frustrating occupation, as Alan Goodall shows in *The Wandering Gorillas* (Collins: London; £6.50). The book is mainly about the wanderings of the author in central Africa and the difficulties of life in the forest although we do get some occasional glimpses of the elusive gorillas themselves. By behaving like a gorilla, chewing leaves and beating his chest, Goodall was sometimes able to observe them at close range. On other occasions he experienced the terrifying bluff charges where a male would stop his advances only a couple of metres away! The gorillas are threatened by extinction, partly from local superstition (one group of six was stoned to death) but mainly by destruction of their natural habitat. Alan Goodall concludes with a plea for international aid to enable the central African governments to offset short-term economic losses and leave the forests intact so that these gentle giants are able to continue their wanderings in peace.

Nicholas Davies

Nicholas Davies is a Demonstrator in Zoology and Research Fellow of Pembroke College at the University of Cambridge, UK.

Intricacies of Nature

THERE seems to be no end to the flow of new books by experienced naturalists whose purpose is partly to educate the public in the intricacies of nature and partly to provide a forum for their authors' reminiscences. *A Natural History of Britain and Ireland* by Eric Simms (Dent: London; £6.95) has a flavour of the nineteenth century about it — accentuated by Robert Gillmor's exquisitely drawn vignettes at the head of each chapter. The author describes the very varied countryside of Britain in terms of its history and of its geographical differences. The book is divided into 15 chapters each of which deals with a different region that typifies a distinctive type of habitat. The book is also largely biographical, recalling the author's many experiences of wildlife in different parts of Britain. It does not make very easy reading — many of the accounts of specific localities, such as the Cheddar Gorge, consist largely of lists of species that the author has seen there. So much information is presented that the underlying theme of the book, an analysis of the factors that have produced the natural history that we see today, is frequently submerged.

Also by Eric Simms, *Wildlife Sounds and their Recording* (Elek: London; £5.95) goes in great depth into one aspect of natural history study, the recording of animal sounds. The book deals with detailed techniques and descriptions of recording equipment, the history of wildlife sound recording and something of the biological significance of auditory communication in animals. There is much interesting information about techniques and about the kinds of sounds that animals make. Unfortunately, the chapter on biological aspects of animal sounds is rather dated. In particular there is little mention of the highly fruitful use of playback experiments in which recorded sounds are played to animals in the field. This has been notably successful in increasing our understanding of the nature of territoriality in birds, competition between red deer stags, and mating strategies among frogs and toads, where synthetic sounds have proved very useful. The book contains a number of interesting photographs, particularly those of early recording equipment.

Encounters with Nature by Leslie Brown (Oxford University Press: Oxford; £6.50) is more revealing of the author than informative about wildlife. Consisting of a series of essays about encounters with animals that the author calls "golden moments" in his life, there are chapters on the aardvark, badgers, flamingoes and pelicans among others, seen mostly in East Africa and Britain. Some of the

observations of animals in their natural state are evocative, exciting and informative but too much of the book is devoted to the author's opinions about the state of the world as he has found it during his life. The book is introduced by a series of contemptuous remarks about "scientists" and is punctuated throughout by comments that reveal that Leslie Brown yearns for the good old days of the British Empire when natural history was a hobby for a few privileged amateurs. While reading this book one inevitably finds that its rather sour, misanthropic tone contrasts with Gerald Durrell's much more amusing and sympathetic treatment of humans as well as animals. The book includes a series of attractive drawings by Doris Tischler.

In marked contrast to Brown's book, with its emphasis on more exotic and spectacular forms of wildlife, Ken Hoy's *On Nature's Trail* (Mitchell Beazley: London; £6.95; ALW: New York; \$14.95) brings us back to the minutiae of wildlife in our immediate surroundings. This excellent and attractive book is a practical guide, telling the reader what to look for, where to find it and how to interpret what we find. It tells us what we can learn from animal droppings and tracks, what we can do to help injured or orphaned animals, how we can appreciate the complexity of life within a single tree, and much more besides. The book is generously illustrated with excellent photographs. As well as

making a very good companion for any young enthusiast, this book should prove useful to teachers in search of ideas for nature trails and school field projects.

Another immensely useful book is *A Day in the Country* by John Gooders (André Deutsch: London; £5.95). This provides a pocket guide to naturalists and more casual observers of wildlife, telling them what they might see in any part of Britain. The book is divided into two parts. Part 1 lists all the National Parks, Forestry Commission areas, RSPB reserves, and many other official localities. Clear maps and lists of addresses make the planning of visits to such places a simple matter. Part 2, which takes up the greater part of the book, deals separately with eleven areas that together cover the whole of Britain. For each area there is information about footpaths, areas of outstanding natural beauty, wildlife parks and zoos, stately homes and many other places of interest to the naturalist. For each locality there is a list of the more interesting and unusual animals and plants that the visitor might expect to see. Illustrated by a number of very attractive sketches by David Thelwell, this book makes a truly valuable contribution to the enjoyment and appreciation of Britain's wildlife.

Tim Halliday

T.R. Halliday is Lecturer in Biology at the Open University, Milton Keynes, UK.

Wildlife of Europe

THE quartet of books reviewed here ranges from the broad canvas of the classification of the Animal Kingdom to narrower studies on groups and single species of carnivores. Naturalists will find the first useful, the rest diverting as well as informative.

Mammals: Their Latin Names Explained by A.F. Gotch (Blandford: Poole, UK; £5.95) clothes the dry bones of taxonomy for the benefit of those without a classical education. The scientific names of animals are, on the whole, indicative of their appearance, provenance or discoverers, and much is hidden from those who cannot derive the roots from which the names are composed. Thus the New World cottontail is known to zoologists as *Sylvilagus floridanus* which tells us that it is a 'woodland hare from Florida'. English names vary locally and lack the diagnostic precision of the scientific name.

The author of this book deals first with the phyla and subphyla of the whole Animal Kingdom from *Amoeba* to man, then with the vertebrates, down to orders in the case of mammals, and finally — the bulk of the book — with mammals down to families and selected species. The principles of nomenclature are explained,

although it is surprising that the importance and adequacy of the first published description of a species are not more emphasised. At every step the Latin or Greek derivation of names is carefully expounded and — particularly valuable — a biographical note is given about explorers and scientists, after whom many animals are named. There are a few misprints and the odd contentious derivation; for example, I do not believe that the name *Didelphis* for the opossum refers to the pouch as a "secondary womb" (page 37); surely it indicates the double nature of the female reproductive tract in marsupials. In any case *Didelphis* does not have a pouch.

This book is a mine of information and illumination to naturalists who have used these names for years without understanding their import.

Carnivores of Europe by Robert Burton (Batsford: London; £8.50) is a timely evaluation at second hand of the status and prospects of the terrestrial, carnivorous mammals of Europe. These most interesting animals have been subjected to intense persecution by man, both as competitors for his domestic stock and as providers of warm and elegant clothing. Now that predatory animals are acknowledged to play a salutary rôle in the economy of nature, Mr Burton's book, giving up-to-date results of recent researches, helps us to assess probable future trends in numbers, both down-

Eris Simms with reflector microphone

Taken from *Wildlife Sounds and their Recording*

wards, due more now to fragmentation of habitats than to direct killing, and, surprisingly, upwards because of ill-judged introductions — for example, American mink and raccoon dogs. In addition, we have to face the possibility that extremely adaptable species, for example, the fox, are now flourishing in urban and suburban environments and could become a menacing carrier of rabies, which would be far more serious than the presumed agency of the badger in transmitting bovine tuberculosis.

The predator which seems to have the least hope of avoiding ultimate exter-

mination, is the wolf. B.H. Lopez in his book *Of Wolves and Men* (Dent: London, £7.95) has produced an impressive and extensively documented survey not only of the natural history of this species but also of the significance of the wolf in hunting societies and of the bitter enmity that exists to this day between it and pastoralists. Most of the book is the outcome of tireless research among the literature but the author has been at pains to spend long periods in the field with Esquimaux in Alaska and to come to an understanding of how wolves are an intimate part of their world. If the wolf has to go, then this book will compose a worthy obituary.

Lastly, we have *Lutra, the Story of an Otter* by Philip Wayre (Collins: London; £3.95). This is the latest in the series *Collins Animal Lives*, each written by a specialist but with a presentation and an outlook which make easy reading for the layman and children. Because the otter is a declining species in England now, much sound and fury has been raised by protectionists to abolish otter hunting and to declare it a protected animal. The results of recent fact-finding surveys suggest that powerful causes of the otter's decline are pollution of our waterways and the increasing pressure of man's activities on them; in which case the establishment of the Otter Trust in Norfolk, of which Mr Wayre is Honorary Director, and the creation of quiet areas as refuges hold out the best hope of rehabilitating this very



Common hamster

Taken from *Animals and their World*

attractive animal. Mr Wayre's little book, which describes a year and a bit in the life of a bitch otter in East Anglia and is enlivened by the delightful sketches of John Edwards, will make many of his readers wish to do their bit in conserving the species.

H. N. Southern

H. N. Southern was until his recent retirement Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford, UK.

Seal saga

DURING the past century the grey seal has increased in numbers in British waters mainly because of the decrease in seal hunting by man. In the same period the status of most stocks of commercially valuable fish in the seas off the Scottish coasts, where the grey seal is most numerous, have declined due to overfishing by man. Seals and man both eat fish, but the quantity and quality of the seal's diet is, for a variety of technical reasons, inaccurately known. Assuming a grey seal eats 7 kg fish daily there is a postulated loss of some 56,000 tonnes per annum to the British in-shore fishery, estimated at £15 million. Unfortunately, the science of both the fishery and seal conservation aspects of the issue are riddled with assumptions and untested hypotheses.

These are the ingredients of a roaring conflict which has arisen and has already been staged between fishery and nature conservation interests in Orkney and North Rona during the breeding season of grey seals in 1978. A thoroughly objective review of the whole affair is difficult to obtain, firstly because of the formidable dossier of evidence and often conflicting

expert comment and secondly, because of the emotive aspects of killing animals of such immense human appeal as seals. Nonetheless, in *Seal Cull: The Grey Seal Controversy* (Penguin: Harmondsworth, UK; 95 pence), John Lister-Kaye has come close to providing an account which is fair to both sides.

Lister-Kaye has provided a first class documentary of the grey seal-fishery issue in which he has systematically presented the information in a form which is useful to statesman, politician, scientist, fisherman, conservationist and interested layman alike. He writes not as a completely detached observer and is not afraid to state

his own opinion on main issues and attribute blame or praise where he thinks fit. He also gives thought to the primeval roots of the problem in 'man and dog' relationships and very sensibly relates the conflict to the international scene and to harp seal harvest in Labrador from which the grey seal issue in Britain derives impetus. A set of personal interviews with prominent personalities shows them to be to a great extent in ignorance and disarray on major aspects of the controversy.

In conclusion there is a personal view in which the author, though hard hitting in some respects, does not damage his balanced treatment. The book does nothing but good in suggesting guidelines by which a compromise in grey seal conservation can be achieved.

In 1978 Orkney was a focus of national public interest because of the cull of grey seals there and at North Rona. In the arena Greenpeace and a local action group called 'Selkie' (Orkadian for 'seal') were pitted against Norwegian seal-hunters who were the contractors brought in by the then Secretary of State for Scotland, Bruce Millan. For every contestant in the field, there was at least one media man. The prospect of shooting, blood and confrontation brought the reporters and photographers in a swarm to Kirkwall.

Sue Flint, a seasoned campaigner for good causes in Orkney, was a moving spirit in the local Selkie group and painstakingly

Taken from *Seal Song*

wrote a diary of events which appears in *Let the Seals Live!* (Thule Press: Sandwich, Shetland; £5.50). This is the story from the Selkie side of the action which, understandably, portrays their action as verging upon the heroic. It is primarily about people, secondarily about seals. If Mrs Flint did not write with such a light touch and with such humour and feminine charm, the book could be a tedium of trivia enlarged to assume an importance far beyond reality by the ambient tension which gripped Orkney at the sight of the Norwegian sealer, *Kvitungen*, closely shadowed by Greenpeace's vessel, *Rainbow Warrior*.

However, much more can be read from this book than a faithful account of a local and national campaign to save the Orkney and North Rona seals from slaughter. It shows clearly the delicate balance which is struck between the rational behaviour of ordinary people and that of the same moving on a sense of bitter outrage. It also shows that the same local people can expect strong national and international support, not just in words but in action on the spot, in very difficult conditions. It also shows how extended, exhausted and near to collapse was the local expeditionary force. The book has many mistakes and shows all the signs of having been rushed. It is, however, something of a latter-day Orkney saga — both informative and entertaining.

Seal Song (Allen Lane/Penguin: London and Harmondsworth, UK; hardback £4.95, paperback £2.50) is a sensitive portrait of the harp seal created in words by Brian Davies with photographs by Eliot Porter. Davies is the executive director of the International Fund for Animal Welfare which has been, through his personal energies, responsible for the campaign against the annual hunt mainly of harp seals off Newfoundland and Labrador.

The book, produced in 'coffee-table' style, described the author's commitment to the battle against the cruelty inflicted by mankind upon wildlife for economic gain. He finds in the harp seals the quintessence of natural beauty, evolutionary adaptation and a subtle psychological, perhaps mystical relationship between man and beast. His reactions are instinctive of a person who is repelled by any sense of pain or bloodshed and there could not be a more vivid subject for such reactions than the slaughter of the fluffy, white seal pups and their sleek, silken dams in the crazy, white world of the sea-ice.

Inevitably, therefore, this is an account which is heavily charged with emotion; while providing some salient scientific information and assessment of the social and political issues, the author makes a direct case for the cessation of the seal hunt on grounds which are mainly humanitarian. His fears for decimation and possible extinction of the harp seal are, however, very real when seen against the potential for exploitation embodied in a

sealing vessel such as that shown in this book.

There are compelling descriptions coupled with excellent full-page colour photographs of the harp seals both on the ice floes and in the strange phantom light in the sea under the ice. Porter's photographs provide superb contrast in texture; the soft, downy warmth, black muzzle and watery, wide-awake eyes of the pups seen against the hard angular shapes of the sea-ice.

The book concludes with a personal account of efforts made by Davies and others against great odds to fly the world's press to the scene of the hunt, in which he risked his life. An outstanding piece of publicity against the seal hunt, it is short, easily read, lavishly illustrated and has instant impact.

A seal emerging from the sea is like a stranger from another world; yet for a few crucial days each year the grey seal must come ashore above high water mark to give birth and mate. They are then so vulnerable to hunters and available for study by scientists. In the 1930s the late Sir Frank Fraser Darling started the scientific work in the Hebrides and this was continued after the war by Dr Leo Harrison Matthews and his friends Professors E.C. Amoroso, Richard Harrison and the late Humphrey Hewer. They became fascinated by the behaviour, reproductive anatomy and physiology of both the grey and common seal and spent many enjoyable days in the

field and laboratory piecing together their biology, particularly the evidence of delayed implantation of the blastocyst, possibly related to the spring moult in the grey seal.

The story is told in the style of a biographical novel by Harrison Matthews in *The Seals and the Scientists* (Peter Owen: London; £7.50). A series of conversation pieces with his zoologist companions in strange places are interspersed with contrasting descriptive sketches of the Welsh Islands and the Wash. There are some exciting moments in coming face to face with the heroine — a grey seal cow called Mrs Mopp!

The discussions of the academics are full of accurate scientific information presented systematically in informal guise. However, the book deals only with the early years of a period of great expansion in seal biology in Britain and the relationship of seals to sea fisheries. That a book of this title and character should have been published in 1979 without greater mention of the seals and fishery issue and some of the main personalities (because it is about people as well as seals) is a great pity. Though sprinkled with many technical and scientific terms, the book will be enjoyed by both naturalist and layman.

J. Morton Boyd

J. Morton Boyd is Director for Scotland of the Nature Conservancy Council, Edinburgh, UK.

Living with cetaceans

SCIENTISTS have seldom been able to study living cetaceans directly, but have usually had to work like detectives building a story either from clues washed up on the beach or from inspecting victims of the whaling industry in order to infer something of their lives from corpses. Unfortunately, the popular tradition of celebrating the careful deductive approach of the detective hero has not become established among popular books on cetaceans. Instead, the cetacean literature has suffered from authors who have taken advantage of the lack of knowledge about these mammals to speculate freely and to present these speculations as the truth.

Whales, Dolphins and Porpoises by Ronald Lockley (W.W. Norton: New York; David and Charles: Newton Abbot, UK; \$14.95, £6.95) is a case in point of this unfortunate tendency. The book is written in a careless, chatty style and its organisation is confused. Many sections seem to have little to do with their headings. For example, the section entitled "Anatomy and physiology" concentrates on cetacean intelligence, buoyancy, diurnal rhythms and sleep. The rough draft quality of this book is by no means as harmful as the fact that many statements

are either confused or false; this problem is aggravated by the fact that not all references quoted in the text are listed in the bibliography.

Lockley's evaluation of the cetacean literature is typified when he maintains that dolphins can probably perceive our thoughts by telepathy and that baleen whales are known to echolocate; or when he states: "They [cetaceans] display almost every human emotion you can name — in short, cetaceans are almost human." Lockley commits enough serious errors of fact that I cannot recommend this book to anyone who wants to be able to distinguish what is known about cetaceans from what might be true or is simply false.

This is a shame, for the book contains many beautiful and interesting photographs as well as a useful set of descriptions and illustrations of most species of cetaceans. Lockley's account of interactions between humans and cetaceans, both cooperative and predatory, is detailed and is much more sound than his attempt to describe cetacean biology.

Wake of the Whale (Hutchinson: London; Friends of the Earth: New York; £15.95) is not a book about whales, but rather describes the work of one of the finest photographers of whales, Bill Curtsinger. The book is organised around a collection of superb photographs taken

by Curtsinger of marine mammals around the world. The large format of the book enhances the fine reproduction of these photographs, which comprise half of the book. Wild cetaceans are among the most difficult of photographic subjects, and this difficulty tells, for the pictures of cetaceans in this book cannot compare with those of seals — either in composition or clarity. In part because of these difficulties, this collection of photographs is eloquent evidence of the persistent and devoted skill of Curtsinger as a photographer.

The text accompanying these photos exhibits a curious ambiguity of purpose. Not only does the author, Kenneth Brower, describe Curtsinger's adventures with cetaceans, but he also devotes equal attention to details of Curtsinger's personal life. The book does contain many statements about the biology and natural history of whales; these are occasionally in error and are tossed off carelessly as if the facts of whale life are peripheral to the

main theme of the book. Brower is obviously fascinated with the issue of what kind of person would devote his life to living on the remote edges of the Earth, constantly struggling for fleeting encounters with marine mammals. Yet Brower never directly acknowledges this hidden agenda of the book and leaves me wondering about his purpose. Does he simply want to extol the virtues of whales as seen through Curtsinger's eyes and camera, or does he want to say something more general about the importance of wildness and mystery in all of our own lives? In spite of this ambiguity, *Wake of the Whale* should interest anyone who wants a beautiful collection of marine mammal photographs accompanied by a description of what it is like to live near these animals.

Peter Tyack

Peter Tyack is a graduate student in animal behaviour at Rockefeller University, New York.

World fisheries

Food from the Sea. By James Nicolson. Pp.205. (Cassell: London, 1979.) £6.50.

THIS book, which is written primarily for the layman and the fisherman, begins with a brief account of aspects of the marine environment which are of importance to fisheries and goes on to consider some of the notable features of fish populations such as reproduction, migration and short- and long-term fluctuations in stocks. This is followed by a chapter which describes some of the main food organisms harvested from the world's oceans. Obviously it is impossible in a book of this size to deal with every important species but in many respects I felt that there was a certain lack of balance in this chapter. For example, the five species of Pacific salmon fished by five nations are only mentioned in passing, whereas the three dogfish of European waters, only one of which has any commercial value, have two paragraphs devoted to them.

In chapters 3 to 7 the author traces the development of the world's fisheries from the earliest times, through the astonishing technological advances of the last hundred years, to the present day. These chapters are well researched and the historical account, laced with many interesting anecdotes, clearly shows that "the history of fishing is a story of human ingenuity and remarkable technological achievement — marred by carelessness and a wanton misuse of some of the richest sources of food in the world".

He then considers the question of overfishing and describes how the development of fleets of ocean-going freezer trawlers in the 1950s and 1960s had a devastating effect on many of the worlds already over-

exploited fisheries. In many cases it was the industrial fisheries which suffered most and as soon as one fishery became uneconomic the fleets simply moved on to uncontrolled exploitation of yet another resource. He then deals with the systematic destruction of the north-eastern Atlantic herring fisheries and discussed the conflict between the traditional fishery on the adult stock with the fishery on the young fish on their nursery grounds for reduction to meal and oil. He then traces the history of the scientific basis for fisheries management and the setting up of the various international organisations with responsibility for conserving the stocks. He is critical of the scientists, the International Council for the Exploration of the Seas and the North-

Eastern Atlantic Fisheries Commission for their failure to conserve the herring stocks.

The author is undoubtedly aware of all the problems involved in the management of a stock but it is unfortunate that some space could not have been devoted to considering the differences between growth overfishing and recruitment overfishing. The latter is an important factor in herring and indeed many other pelagic species which grow little in their adult lives, and the failure of the scientists to understand the relationship between recruitment and stock size was a key factor in the collapse of the herring fisheries.

Looking to the future the author considers the role of fish farming as a means of increasing food production from the sea. Throughout the book one gets the impression that, while not denying that fish meal production has a role to play in utilising the resources of the seas, the author would prefer to see a greater emphasis on the use of fish for direct human consumption. It is therefore surprising that he is not critical of fish farming as practised by most of the developed nations in which fish meal is used to produce luxury protein in the form of species such as salmon and trout.

I have no hesitation in recommending this book to anyone with an interest in the fishing industry, especially to anyone who wishes to understand the background to the present problems facing the industry and the controversy involved in the formulation of a common fisheries policy within the European Economic Community.

J.D.M. Gordon

J.D.M. Gordon is a Principal Scientific Officer at the Dunstaffnage Marine Research Laboratory, Oban, Argyll, UK.

Sea guides

Marine Life, by David and Jennifer George (Harrap: London; £16) is an extremely well produced book describing marine invertebrates. The centre section of the book consists of 1,300 colour photographs of high quality, while the two end sections describe the various invertebrate phyla down to family and a "representative species". There are a number of line diagrams in these sections of the book. Technical terms are reduced to a minimum and a short glossary is provided. Nevertheless, readers without zoological knowledge might find the book a little daunting. It is not unlike some of the modern gardening books, with a centre section of colour plates and a fairly technical description at either end.

Although the publishers claim it is an encyclopaedia, it could not be used for identification of different groups down to species. Nor could it be considered as "an

indispensable work of reference." It is true that scuba diving has enabled biologists and laymen to see animals in their natural state but detailed identification is still done from fixed specimens even if they are "distorted and colourless on the laboratory shelf." The authors seem to take a rather simplistic view of identification, although David George is on the staff of the Natural History Museum. I agree with the Foreword that it is a fine book but it is not a field guide in the sense that it could be put in one's pocket. It is excellent value for £16 and worth a place on the library shelf of any institution, or anybody interested in marine invertebrate zoology, but it should fulfil the role of a colourful adjunct to a systematics course rather than a substitute for the rigours of formal systematics.

Kenneth Gosner's *Field Guide to the Atlantic Seashore* (Houghton Mifflin: Boston; \$12.95) would just fit into a capacious pocket. It lists 907 littoral invertebrates and 140 seaweeds from the NW Atlantic from the Bay of Fundy to

Cape Hatteras, although some species extend beyond these limits. The centre section consists of 64 black-and-white and colour plates which are drawings of the species; the two end sections consist of descriptions of the species with notes on seasonal occurrence and where they are to be found. There is no attempt to divide the species within phyla. At the beginning of the book there are introductory chapters on how to use the book, nomenclature, collecting and preserving specimens, labelling, distribution and habits and zonation, and at the end a glossary. Such a book must be of limited use to collectors on British or European seashores, especially

when goods books are already available (see *Nature* 264, 587, 1976).

David Bellamy is well known as a botanist-cum-broadcaster. His book *Half of Paradise* (Cassell: London; £7.95) bears no resemblance to the other two; it is a breezy and well illustrated account of two Joint Services underwater expeditions to the Chagos Archipelago in the Indian Ocean between 1972 to 1975. They appear to have been a combination of a moderately serious scientific enterprise, a Services training exercise and for gathering material for a BBC documentary programme. Results were obtained on the growth of coral reefs, migration and breeding of

seabirds and on the flora and fauna of the islands. The expedition charted the islands, dived on wrecks, had brushes with sharks and robber crabs, rats, mosquitoes and sand flies and generally had an eventful time. The book has a Foreword by Prince Charles, who clearly would have enjoyed being a participant. This is a book worth borrowing from the public library if one can 'take' David Bellamy's mildly irritating style of writing.

J.H.S. Blaxter

J.H.S. Blaxter is a Senior Principal Scientific Officer at the Dunstaffnage Marine Research Laboratory, Oban, Argyll, UK.

Fish guides

Two recent guides to British and European fishes could scarcely offer a greater contrast. Judging from their titles and general appearance, L. Cacutt's *British Freshwater Fishes: The Story of their Evolution* (Croom Helm: London, £6.95) is a serious and (one anticipates) authoritative study of the evolution and biogeography of Britain's freshwater fishes, *Fishes: Freshwater and Marine Species* (Chatto and Windus: London; paperback £2.50) second is slighter, being a collection of colour photographs with modest text fitted into matching spaces opposite the picture. One is written by a journalist professionally interested in fishes, the other by an Austrian ichthyologist and translated and adapted by an English zoologist. The first is pretentious, filled with errors and fails in its objective of presenting a coherent account of the fish fauna; the second is as authoritative as its appearance is modest.

Terofal's little book, a *Chatto Nature Guide* contains approximately 120 colour photographs of living fishes, mostly taken in aquaria. The standard of photography is high and the colour reproduction good. Most of the European freshwater fishes are included but the coverage of marine species is less good. Colour photographs of many of the Danubian species have not been reproduced before in English texts and this is a strong point in the book's favour. The text is organised in systematic order, and for each species included there are short, rather telegraphic, accounts under headings of, characteristics, distribution, habits, and diet, although the use of these headings is not consistent throughout. Errors are few; for example, the nine-spined stickleback has been placed in the genus *Pungitius* for several years but is here referred to as *Gasterosteus pungitius*, and the photograph opposite the caption line, *Molva molva*, is of a torsk, *Brosme brosme*. Such minor errors apart, this is an excellent book and should help fill the stocking of many an angler this Christmas

— and probably those of quite a few ichthyologists also.

Of Cacutt's book I wish I could say the same. It starts with a typographical error on the fifth page of the prelims with "A fossile pike" a standard which continues more or less throughout. "Spiney-finned Fish" are referred to so often that I had to resort to a dictionary to check that it was not a permitted alternative for spiny.

The author's intention is said to be to present "the broad spectrum of the multi-million year-old story of Britain's freshwater fishes". There are also discussions of fossil fishes and some geology, and potted accounts of continental drift and of the ice ages, of which the last has some relevance to the subject. The major part of the book, however, is a collection of accounts, of British freshwater fishes, species by species, with heavy emphasis on anglers' interest in weights and records, and on behaviour.

The author's familiarity with the subject seem sketchy. On the interesting biogeography of the Irish freshwater fishes we are told on page 60 that the bream, loach

and rudd are indigenous to Ireland and then on page 128 that the bream was introduced, while on page 110 the rudd is omitted from another list of indigenous species. In fact, none are indigenous to that island. The author shows a positive genius for getting terms and names wrong, for example, andronomous for anadromous, Roderick Imprey Murchison for R. Impey Murchison, and *Erythrophthalmus scardinius* for *Scardinius erythrophthalmus*. This trait provides a little light relief in places as on page 12 where Darwin's "*Evolution of the Species*" is described as a classic example of a "stodgy" scientific book, "sheer drudgery to read". Alas, Darwin wrote no such book.

This book certainly cannot be recommended, except perhaps as an example of the old saw that a little knowledge is a dangerous thing.

Alwyne Wheeler

Alwyne Wheeler works on fishes in the British Museum (Natural History), London, UK.

Happiness is helical

Two recently published books dealing with aspects of the study of molluscs, they are as different as roast beef and candy floss. *Sea Shells: A Naturalist's and Collector's Guide* (Phaidon: Oxford; £6.50), by Peter Wilson and Patricia Newell, is very pretty; it has a saccharin sweetness about it and it will appeal to shell novices. But such a diet cannot satisfy for long. Although this book contains many colour pictures by famous invertebrate specialists like Douglas P. Wilson and Heather Angel, the captioning is poor. The information given is sometimes inaccurate (the beautiful nudibranch illustrated on page 23 is not even correct to the nearest family), and the provenance of the specimens illustrated is not uniformly helpful. The text is undistinguished and there is on page 42 a "Key to the most

common classes of mollusks" which will confuse any budding shell expert; it should have been left out. *Sea Shells* is a pleasant coffee-table book, no more.

M.P. Kerney and R.A.D. Cameron's authoritative *A Field Guide to the Land Snails of Britain and North-west Europe* (Collins: London; £5.50) in the Collins Field Guide series is the roast beef. It may require more effort to chew and swallow, but the nutritional benefits are substantial. This is the first compendium to deal comprehensively with the land snail shells of the whole of North-west Europe. There are, of course, fairly good guides in existence, dealing with the land molluscs of individual countries, but some of these are out of date, or so massive as to deter even the professional. The present guide describes and illustrates the 279 known species (plus a few stowaways which have turned up in greenhouses) in a way which will satisfy even the most demanding specialist; at the same time it can be used and enjoyed by

those with little or no previous experience.

The land mollusc fauna increases in richness from north to south in the area covered by the guide. Iceland has only 25 or so species, southern Britain about 100 species, and parts of France over 130 species. This kind of faunistic recording is fascinating, and is based upon data accumulated by a vast army of amateur and professional devotees. Outside ornithology, there are few areas of practical biology where the amateur can play a more effective part than malacology, the study of molluscs. The authors give us all the information we need to enable us to join in the fun. There are chapters on the shell, soft parts of the body, life-history,

feeding habits, classification, and an excellent introduction to finding, collecting and preserving land snails. Kerney and Cameron will win the gratitude of all those professionals who routinely identify and handle land molluscs, for surely many young malacologists will be recruited through reading this guide. I should like to see a copy in every haversack, along with the usual maps and ornithological field guides. **T.E. Thompson**

T.E. Thompson is a professional malacologist whose most recent appointment was Visiting Professor of Biological Sciences, Florida Institute of Technology.

For the entomologist of the future

THREE recently published popular books on insects differ a good deal in their scope and treatment of the subject. Professor Karl von Frisch, a Nobel Laureate honoured for his work on bee behaviour, is well known to a wider audience of naturalists for his popular work, *The Dancing Bees*, a book which, to my mind, is very much more interesting than the one now under review. *Twelve Little Housemates* (Pergamon: Oxford and New York; hardback £8, paperback £2.50) is a revised and enlarged version of a book on ten groups of insects found in and around houses, and includes accounts of the house-fly, mosquitoes, fleas, lice, bed bugs, clothes moths, cockroaches, ants and aphids. The species are well chosen and their biology is described accurately and simply, in a way understandable by the complete layman or by a child. The book is hardly detailed enough to satisfy the serious amateur naturalist or the budding entomologist, and the rather forced humour of the text and the Victorian air of many illustrations are not likely to win over a sophisticated reader. But von Frisch is mildly discursive and despite some of the naively phrased passages he is able, in his own words, "to show that there is something wonderful about the most detested and despised of creatures".

Dr Carson Ritchie, an archivist and historian by training, believes, in his *Insects: The Creeping Conquerors and Human History* (Elsevier/Nelson: New York; \$7.95) that insects have had crucial effects on human history and sets out to describe some of these. For better or worse, the book is largely a collection of historical anecdotes, rather brashly presented and strung together in a loosely chronological sequence, on such subjects as silk production, singing insects, insects as human food, and insects in medicine and art. Entomology is not really one of the

author's strong points and the occasional howlers provide additional surprises in a book already packed with stories intended to excite, intrigue, startle, alarm or confound the reader. Entertaining snippets of information abound on every page. Did you know that machinery able to produce 318,504,960 yards of silken thread per day had been installed in Derby in 1718? Or that France imported 57 million medicinal leeches in 1823? (By an elastic definition of the Insecta, Dr Ritchie manages to include earthworms, spiders, palolo worms and *Teredo* among his creeping conquerors.) And there are illustrations of all manner of things from Maori tattooing chisels to what I presume are geisha girls entranced by the music of cicadas. But not all readers will be convinced by claims that the silk industry led to the Industrial Revolution or that *Phylloxera* played an important part in the downfall of Napoleon III. Certainly the scholar will regret the more uncritical passages and the almost complete absence

of references in support of so many facts.

By contrast, *Insects: An Illustrated Survey of the Most Successful Animals on Earth* (Hamlyn: London and New York; £10), by Brian Selman *et al.*, is a straightforward account, lavishly illustrated with more than 200 colour photographs, line drawings and diagrams. No doubt it will be the coloured illustrations which will fascinate the layman. They certainly convey a most vivid impression of the diversity of insects and, by often portraying things much larger than life, they enable anyone to see insects exactly as the professional entomologist views them through a stereoscopic microscope. The quality of reproduction is generally high and some are superb portraits of handsome subjects with gleaming cuticles, quivering wings, and alert activity suggested by every bristle! The text that accompanies these pictures is written by a group of specialists and deals in turn with the evolution and distribution of insects, their structure, physiology and development, their habitats and modes of life, their impact on man, and their classification. All these are discussed in an authoritative, modern way, and without over-simplification. The result is a book which depicts very successfully the great variety and biological interest of the insects. It can be enjoyed by a 14-year-old who is keen on biology, but it will also serve as a reference book for all but the more specialised undergraduate courses in entomology. Not cheap, but a good Christmas present, especially for the entomologist of the future.

R.G. Davies

R.G. Davies is Reader in Entomology at Imperial College, University of London, UK.

Traditional remedies

THE great majority of the world's population is poor and their medicines are traditional, being mainly plant or animal in origin. These people either care for themselves when ill or use traditional healers. In both cases the sources of their remedies are well known, and children learn from an early age which plants can or cannot be used, and for what purposes. It was realised some time ago that the plants used in these traditional forms of medicine might well repay scientific investigation and there are various projects underway (and no doubt afoot) for doing just that. This is a long way, however, from the current fad for using herbal remedies instead of modern pharmaceutical products. The fashion seems to be confined to the English-speaking world, no doubt because the inhabitants of mainland Europe have never stopped using

traditional remedies for a variety of conditions. This may well be due to the fact that there are still peasants in these countries who know and collect the plants. Would that we had a reasonable reservoir of people here who could do the same, but I am afraid this is not the case. This raises a very important point. The books reviewed here are designed for amateurs, not professional botanists and plantsmen. It is essential that either the plants listed should not be harmful or the descriptions so detailed and clear that mistakes should not occur, within the limits of human fallibility. This is especially important when describing members of the Umbelliferae (parsley) family, most of which look alike to the layman and include both very useful herbs and extremely poisonous plants.

Nicholas Culpeper's Complete Herbal and English Physician (Gareth Powell: London; £15) is a facsimile of the 1826 edition but was first published in the seventeenth century, when medicines were

based on plants and folklore. The plants are listed alphabetically under common names, with a description of uses, habitat and time of harvest. Culpeper also gives the sign of the zodiac with which each plant is associated and the effect this has on its use. He was greatly criticised in the past for his interest in astrology — now only of historical interest. He also lists the temperament of the herbs — hot or cold — and the effect this has on the body. There are sections on gathering and drying the herbs, and on the preparation of medicines. The section of cures for various ailments are not all of plant origin; if you rub black snails on a wart and then stick the snail on a blackthorn twig, the wart will go. The archaic English makes this book difficult to read; and although the illustrations are pleasant, its value is historical rather than practical. A good present for devotees of the past.

Maurice Mességué is a practising herbalist, much of whose knowledge is a family inheritance. His book, *Health Secrets of Plants and Herbs*, has been translated from the French (Collins: London; £7.95) and is somewhat flowery in style but down to earth and practical in content: he knows and uses the plants he writes about. He sensibly keeps the list of plants to a hundred readily available ones, enough as he says for the normal needs of a family. He also omits nearly all those with poisonous or irritant properties; and where they are listed, he is careful to say how they

should or should not be used. Reading this book has been a pleasure, even if one is left feeling that intestinal worms are rather prevalent in southern France. One matter which will not meet with complete approval in this country is the author's use of garlic practically as a cure-all. The use of garlic plasters for warts and corns (they work within two weeks) beggars the imagination.

Keith Vincent Smith, the author of *The Illustrated Earth Garden Herbal* (Elm Tree Books/Hamish Hamilton: London; £4.95) is an Australian journalist turned self-sufficiency gardener and writer. His book, described as a herbal companion, is an historical account by various ancient and fairly modern writers, of forty herbs, their "virtues" and uses, with recipes. This is an attractive work, of no great practical use, but obviously compiled with knowledge and affection for the subject. There are a few small errors — the genus *Tropaeolum*, for example, is a member of the

Tropaeolaceae not *Cruciferae*.

The Encyclopaedia of Herbs and Herbalism, edited by Malcolm Stuart, (Orbis: London; £12.50) is disappointing. It is a pleasure to look at, but tries to cover too wide a field — not possible in the space available. There are too many illustrations — not all relevant — and some duplication of information. The quick gallop through the plant kingdom could have been omitted, as could that through the human body. The food recipes too are unnecessary. The main alphabetical list of herbal plants is well done but there is not enough general emphasis on the care needed in identifying and using such plants. This is the most ambitious of the four books under review, but the least successful.

Rosemary Angel

Rosemary Angel is Officer-in-Charge of the Museums Division at the Royal Botanic Gardens, Kew, UK.

Nature's jewels

YET more to add to the spate of books attempting to satisfy what publishers have found to be a very rewarding money-spinning exercise these days. Fungi have now come of age, and mushroom and toadstool books are now respectable enough to sit on the book-shelves along with those on flowering plants. It is as if the public in all walks of life have suddenly found the lost chord and whether the books deal purely with collecting and looking at fungi or eating them, or some kind of mixture of both people's appetites are apparently insatiable. Hopefully in the future the public will become more discerning as many of those illustrated texts now available are misleading.

It is refreshing that the present publications under review form a considerably better trio. Each is very different in its approach although inevitably they cover much common ground. There is still the need for a good book on mushrooms and toadstools that bridges the gap between the coffee-table approach and the advanced treatise although these present books partially fill that gap.

Geoffrey Kibby's contribution, *Mushrooms and Toadstools: A Field Guide* (Oxford University Press: Oxford; Hardback £7.95, paperback £4.95) consists of fifty pages of introduction, text, the major part of which is a key, and glossary, and two hundred pages of descriptive data accompanying illustrations. The illustrations are all from original water-colours (by Sean Milne) and seem to have been accurately taken from nature; the colour reproduction has been fairly faithful although the species depicted have

a somewhat more lustrous appearance than when seen in the field. The abbreviated descriptions are from Kibby's field experience and so have some credibility. Over four hundred larger fungi are depicted, some of which are rare in Britain and are illustrated for the first time in a British field-guide. The key (with novel colour-coding) introduces the reader to the up-to-date classification. Usage by the uninitiated will only show how useful this approach is over more conventional keys.

E. Soothill and A. Fairhurst's *The New Field Guide to Fungi* (Michael Joseph: London; £7.50), is from the illustrative point of view the other side of the coin; the text by Fairhurst being supported by 252 coloured photographs by Soothill. Some of the latter seem to have been rather poorly reproduced, and some of the subjects have been partially burnt out by the flash technique used. Although the author has field experience, the descriptions in the majority of cases are taken from other field guides or monographic treatments, especially the microscopic data; and some unfortunately are incorrectly determined, for example, *Lepiota sistrata* and *Mycena pura*. The format is so very open that a lot of valuable space is wasted, and by using the same size of photograph, whether tree or minute fruiting body, it is difficult to judge size. In order to appeal to a larger audience some microscopic fungi are included as well as a spattering of North American species, for example, *Polyozellus multiplex*. Both groups could have advantageously been left out, as their inclusion, especially the New World species, detracts from the workability of the key which somewhere in its production has gained the wrong numerals (page 24 read 18 for 17; page 26 read 57 for second 37). The introducing text is quite short with an accompanying glossary and poor line-drawings; two indices are included. The



Taken from *Tacuinum Sanitatis in Medicina* (14th century)

book does not stand up to its dust-jacket statements suggesting that photographs are greatly superior to coloured paintings, as comparison with Kibby's book will soon show.

The Encyclopaedia of Mushrooms, edited by Colin Dickinson and John Lucas (Orbis: London, £9.95), is an interesting mixture of coffee-table style and scientific approach, and contains a mine of information as one might expect from the title. It needs several readings before the full extent of the details, from eating to electron microscopy, and chemistry to ecology, can be appreciated. The text of 280 pages is largely given over to descriptions with an unfortunate mixture of photographs and often appalling coloured illustrations some of which do not even resemble the fungi they are said to depict. The photographs gleaned from various sources are as might be expected of varying quality depending on the photographers and their wide spectrum of techniques. Many of the photographs are of species which have never been seen in a British publication before and so it is refreshing to get away from the same 'old' photographs of fungi, often misidentified, that one sees in the numerous books now available. As in Soothill and Fairhurst the descriptions are short; many are taken from the literature, as again they do not tie-up with the illustrations and therefore the field collections. Over five hundred larger fungi are illustrated with four hundred and thirty by photographs; unfortunately there are some mistakes, for example, *Mycena pura* has become *Laccaria amethystea*, and so on.

The introduction is a good account of the fungi and makes easy reading for the interested student. It is supported by very well chosen illustrations, and the ecological approach of many sections is admirable. Dickinson and Lucas obviously call on their joint teaching experiences, as they have brought together many of those interesting biological phenomena exhibited by fungi with which the layman is unfamiliar. As the dust-cover says, the *Encyclopaedia* is a combination of field guide, natural history encyclopaedia and cookery book. Indeed it is, Dickinson calling on his own culinary interests for the last.

Each book is well bound and on good quality paper; the printing is clear. Some mis-spelling of latin names is unfortunately inevitable and Soothill and Fairhurst's contribution is worst for this — even the dust-cover has one!

Whereas Kibby's contributions could be tucked into a 'poacher's pocket' and taken into the field; Soothill and Fairhurst's book is an awkward size; and Dickinson and Lucas' production was obviously designed for use in the home and library.

Roy Watling

Roy Watling is a Principal Scientific Officer and Senior Mycologist at the Royal Botanic Garden, Edinburgh, UK.



Illustration taken from *The Tree*, published by Aurum Press (London, 1979; £9.95). The text, by novelist John Fowles, accompanies some beautiful photographs by Frank Horvat. Fowles "explains the impact of nature on his own life, and warns urban 'civilized' man of the deeper and more subtle dangers that attend his traditional rejection of the wild."

Arboreal devastation

After the Elm, edited by B. Clouston and K. Stansfield (Heinemann: London; £7.50) is described as both a requiem for the elm and a guide to the future care and improvement of the countryside. The first chapter, by Brian Clouston, describes the history of the elm in Britain with an emphasis on its role in the landscape. Reference is made to the works of John Evelyn and Gilpin, and there are quotations from Tennyson, Thomas Hood and Herbert Read. My only criticism is that the author persists in describing the wych elm (*Ulmus glabra*) as an introduced species, while as Eddie Kemp makes clear in the next chapter "The Plantsman's Elm", it is unquestionably native. Kemp himself wisely avoids becoming embroiled in controversies over elm taxonomy and presents a straightforward description of the principal types of elm to be found in Britain. Many of the photographs illustrating the trees are, however, disappointingly dull.

Kathy Stansfield's description of "Elm in the Service of Man" overlaps somewhat with the content of the first chapter, but also describes the use of elm wood in the construction of carts, pumps, furniture and boats. Then follow two useful chapters on the biology of Dutch elm disease (D. A. Burdekin) and on the impact that the disease has had on our elm populations (Peter Jones). While appropriate reference is made to methods of disease control, I would have liked to have seen some allusion to the important tree populations which have so far been preserved by control programmes. The Huntingdon elms of Hove park and the magnificent English elm around the Brighton pavilion deserve to be made the object of pilgrimage.

The last third of the book is devoted to a

discussion on tree planting. There are cogent criticisms of some current fashions — for example, that of planting in field corners — and useful comments on the choice of species. Thus the authors point out that the dark cypresses suitable to Italy create in Britain an effect of "gloomy walks and green-slimed stonework". There is some speculation on the extent to which the current enthusiasm for wood-burning stoves could lead to a renewed interest in coppicing as a system of woodland management, and also a welcome emphasis on the necessity for the adequate maintenance of newly planted trees. In summary, although less visually exciting than Gerald Wilkinson's *Epitaph for the Elm* (Hutchinson: London, 1978; Arrow paperback 1979; £4.95; for review, see *Nature*, 276, 641; 1978) this is a more satisfactory book. A proportion of the royalties will go to the Tree Council for the National Tree Fund.

Robert Lamb's *World without Trees* (Wildwood House: London; £4.95) is an anguished response to the devastation caused by Dutch elm disease, and there are sections on the elm, on the infection process, and on possible control measures. Interleaved with these are other chapters dealing with huge topics such as the destruction of the tropical rain forests, and the world-wide consumption of wood and wood products. Mr Lamb's racy style is often exhilarating and he has made a commendable attempt to describe the complex story of the disease cycle and the host's response in non-technical language. Sometimes, however, his intoxication with words carries him away: "fungal livestock, usually in the form of spores, may now become smeared into the notch by the carrier beetles jockeying for mouth-holes on the sap-rich wood".

More seriously, a lack of authority pervades the whole text. To take one tiny example he describes tyloses as "loops"; presumably because he has only thought of

them in two dimensions. A few pages later he states that the vertical furrows in the bark of elms "serve to prevent water-logging of the trunk by channelling off storm water". One wonders how smooth-barked trees like beech manage to survive at all! He speculates wildly on the subject of plant pathology. For example, he suggests that the ice age may have altered the course of evolution "by way of wholesale disease epidemics long after the climate itself had stabilised". The current deforestation by man is therefore seen as

"a speeding-up of a natural process which would happen even if humans did not exist". Although one shares his concern over the way in which the world is using its forest reserve, the chapters on this topic fit incongruously into the framework of the book. I doubt if anyone can read it without feeling both breathless and confused.

John N. Gibbs

John N. Gibbs is a Pathologist at the Forestry Commission Research Station, Farnham, UK.

Botanical enthusiasm

SOME books are written to entertain and others to educate. Many books fail to achieve either aim, and a few manage to perform both functions. Naturally, the kind of book which *Nature* selects for review tends to be of the informative type, but even within these one may seek hopefully for a glimmer of aesthetic appeal lurking between scholarly brown covers. The collection of botanical publications reviewed here happily departs from the general run of scientific tomes dissected in these columns and consist largely of books for the amateur, the student or the enthusiast (the three are not necessarily coincident).

In the area of field guides especially, publishers undertake the task of conveying information to a non-technical audience in an accurate yet attractive and pleasing way. Five of the books here may be placed within this group and of them the most novel is *The Audubon Society Field Guide to North American Wildflowers: Eastern Region* by W.A. Niering and N.C. Olmstead (Knopf: New York; \$9.95). This book is novel in a number of respects; first in its format which is small, if fat (almost a genuine pocket book), and its flexible plastic binding. It is evidently designed for hard wear in the field. Second it has a thumb tab on the pages of illustrations which indicates the colour and form of the flowers or inflorescences featured on that spread. Illustrations are arranged primarily on grounds of colour and then upon such characters as symmetry, or its lack, in individual flowers and their arrangement approach to the difficult task of leading the user to a group of species which he may compare with the unknown specimen.

There are, however, many criticisms which can be levelled at this approach. It departs from any strictly taxonomic basis for identification. All but the purist would find this forgivable as long as the characters used eventually permit accurate identification. In this case there are certain problems in obtaining this end. The illustrations, which provide the basis for identification, are colour photographs; however good these may be, it is impossible to portray all those features of the plant

(growth habit, basal and stem leaves, inflorescence and flower structure, and fruit) which could have been more effectively represented by art work. A second criticism concerns the separation of the text from the illustrations. The text is full, informative and written in an informal style; it also refers to many species which are not illustrated. The general coverage (658 species) is only half that of Peterson and McKenny's *Field Guide to Wildflowers* (Houghton Mifflin: Boston; 1968) which remains the best available field guide for the north-eastern United States.

E.N. Kozloff's book, *Plants and Animals of the Pacific Northwest* (University of Washington Press: Seattle and London; paperback £6.30), undertakes the gargantuan task of providing an introduction to the entire natural biota of an area which approaches 80,000 square miles. Obviously the book cannot attempt a comprehensive coverage and it concentrates upon plants (including some bryophytes, lichens and fungi), vertebrate animals (apart from birds) and some invertebrate groups, such as molluscs. The text descriptions are lucid and contain an enthusiasm and casual detail which tell of the author's evident first-hand experience in the field. It is extensively illustrated by colour photographs which are generally of a higher quality than those in the Audubon guide. The coverage is very selective, but it succeeds in conveying considerable quantities of information in a pleasing and attractive manner. It must be highly valued by naturalists in that part of America.

The Oxford Book of New Zealand Plants (Oxford University Press: Oxford; £32.50) by L.B. Moore and J.B. Irwin also attempts a wide botanical coverage, from diatoms to palms. The format follows the familiar pattern of preceding Oxford guides with facing pages of text and plates. It is a very welcome book in that so little is available in the way of illustrated guides to New Zealand plants; and the artwork is both accurate and pleasing. Representatives are selected from major families of plants found in the Islands and are illustrated in considerable detail, floral sections often being included. It is sad that the bulk of the plates are two-tone as the coloured illustrations are quite superb and the book succeeds in being both entertaining and informative. Although

dealing with a flora from the opposite side of the earth, this work should appeal to botanists and horticulturalists in Europe also, for some genera, such as *Acaena* are known as wool aliens and are now established immigrants, whereas others, such as *Hebe* are important decorative genera.

Blandford Press (Poole, UK) have made a brave attempt to fill the great monocot gap and have issued an illustrated guide to *Grasses, Sedges and Rushes in Colour* (£3.95) by M.S. Christiansen. This group of plants is sadly neglected among the current field guides, but I rather doubt whether this particular book will provide what is needed for the uninitiated to identify accurately these tricky plants. C.E. Hubbard's *Grasses* (Pelican, first published 1954) and A.C. Jermy and T.G. Tutin's *British Sedges* (Botanical Society of the British Isles, 1968) are far superior in that they provide keys and in that the illustrations are detailed and accurate. Colour is of little importance for the portrayal of these groups but as distinction often involves details of floral or fruit structure or, in the case of grasses, certain vegetative details, such as the ligule, these need to be clearly visible in the illustrations. In the Blandford paintings one cannot see the hairy ligule of *Sieglingia*, nor the prominent awn in *Holcus mollis* which helps differentiate it from *H. lanatus*. The attempt by the artists to exaggerate the

VOYAGER I

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differences in hair distribution within these two species is not at all successful. Perhaps there is a need for a good field guide to these groups which is less formal and has more popular appeal than the two works mentioned above, but this is not it. When will an intrepid publisher move into bryophytes and lichens, I wonder? The *Oxford Book of Flowerless Plants* (Oxford University Press) has taken a step in this direction, but much remains to be done.

The Audubon Society of the United States has into the coffee-table market with its *Book of Wildflowers* (Harry N. Abrams: New York) by L. Line and W.H. Hodge. This is a large book which combines bigger, better colour photographs (five frontispieces gradually focusing in on a single *Eschscholtzia* bloom 30 cm in diameter), with a blotting paper text, and large print evidently designed for those myopic botanists who get past the frontispieces. As an example of botanical photographic art, the pictures in this book would be very difficult to beat. Every possible photographic technique is used to enhance the glamour of the angiosperm's reproductive structures, which is a perfectly good reason for publishing this book. The text is arranged by habitats, is casual and informal in style, and is reasonably informative. It evidently seeks to convey something of the excitement and appeal of field biology, which is a laudable aim.

Two books which appear to be aiming for the conveyance of information rather than the stimulation of gasps of rapture among their readers are those dealing with the description of flowering plant families. P.H. Davis and J. Cullen have issued a second edition of their key, *Identification of Flowering Plant Families* (Cambridge University Press: hardback £6, paperback £1.95) found wild and cultivated in north-temperate regions — first published by Oliver and Boyd in 1965. Although it is not a subject which any longer receives great prominence in most undergraduate botany courses, classical taxonomy still forms the basis of much field ecology and its neglect is resulting in a generation of students trained in concepts and lacking the skills of field identification, and sometimes even observation. It would be a useful exercise to encourage students to work through a key like this at their leisure, both in natural habitats and in gardens. It should prove a useful supplement to V.H. Heywood's *Flowering Plants of the World* (Oxford University Press, 1978).

R. Headstrom's *Families of Flowering Plants* (Thomas Yoseloff: London; £12.50) lacks the key of Davis and Cullen's book and falls very far short of Heywood's book in its content and presentation. The book contains a discussion of 212 families, including some gymnosperms arranged in alphabetical order of English names, and deals with each family by selecting species of interest and describing their structure and traditional uses. This is not a book for

anyone interested in taxonomy, evolution, biogeography, ecology or morphology.

Is it reasonable that one region of botany has been totally ignored by the entire scientific community simply because of its non-existence? This is a question framed by L. Lionni in his book *Parallel Botany* (Wildwood House: London; Paperback £3.95). Why has it not been reported in *Nature* that the flowers of *Anaclea taludensis* vaporise instantly on contact with any object which is not part of their normal ecological environment (see Kamicochi, *Proc. Int. Bot. Congr. Tsuchimachi*, 1974)? Is this indeed a demonstration of Leibschmidt's law that "for every immobility in time there is a corresponding immobility in space"? If you find this kind of question intriguing, then this is the book for you, as long as you are contented with questions and do not seek answers. A book for the Botany Common Room rather than the library; definitely designed to entertain rather than to inform.

The Story of England's Flora (Kestrel: London; £6.95) by E. Hyams is an attractively produced and lavishly illustrated book. The text leads us on a brief and rather superficial tour of palaeobotany in which the information is reasonably accurate but is so patently second or third hand that it lacks conviction. It proceeds to describe trees, shrubs and herbs (both native and introduced) which have an interest for man. The line drawings are inaccurate (hirsute and crenate *Fagus* leaves), inappropriate (Lombardy poplar in a text piece on the Tertiary) and misleading (a woodland referred to as beech on chalk in which the mature trees are oak and which is almost certainly upon a clay with flints substrate). The book is in part redeemed by some quaint anecdotes concerning the introduction, origin and the breeding of some domestic plants. Another sadly superficial book which deals with a fascinating subject is *Plants that Eat Animals* (Nelson: Nashville and New York) by J.H. Prince. It is a survey of insectivorous plants, their structure, mode of action and physiology. Even though it is intended as a popular book, in the sense that it appeals to the general public, it could have been so greatly improved if the results of modern research had been included. How can one write such a book without including scanning electron micrographs

of the glands of *Pinguicula* and without any detailed discussion of the transfer of 'nervous' impulses in plants? Instead we have a simplistic essay illustrated by crude line drawings.

Any review of botany books which attempt to combine education and entertainment would be incomplete without reference to David Bellamy. Two books have emerged on to the market following his *Botanic Man* television series. The book with the same title as the series (Hamlyn: London; £6.50) is a didactic summary of the ideas presented over the television screens. It contains many double-spread colour photographs (a 40 cm diameter bromeliad competes well with the Audubon spectacular in the battle to blow the mind) but Bellamy puts these diversions to far better use by punching home attractively presented information in the intervening pages. He seems to be aiming for the advanced school audience, but how different his approach from Lawson's *Textbook of Botany*! The range of concepts he covers is impressive (from the Periodic Table, through DNA, nutrient cycling at a global and ecosystem level, vegetation zones in relation to climate, plant productivity, even to island biogeographic theory). The text, like David Bellamy himself, is forceful, concise and energetic; it must be read with a South London accent. But above all, this is a living, enthusiastic book which does not avoid or skim lightly over awkward questions; it meets them head on.

A second booklet, *Botanic Action* (Hutchinson: London; hardback £4.95, paperback £3.95) is literally an activity book for children, giving them ideas on how to design simple experiments at home, such as growing seeds in used tea bags to look for mineral deficiencies. Or, how about making paper from stinging nettle fibres, or leaf skeletons, or plant based dyes, or dandelion tea? If you have active children interested in their surroundings and if you are not too worried about the state of the carpets, this is just the book to give them for Christmas. Then is the time to judge whether a book can be both informative and entertaining.

Peter D. Moore

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences at King's College, University of London, UK.

Guide to alpine flowers

The Alpine Flowers of Britain and Europe. By C. Grey-Wilson. Pp.384. (Collins: London, 1979.) Hardback £5.95; paperback £4.50.

THIS is a most useful field guide to the flowers of the hills and mountains of

Britain and the continent. It spans from Cape North to the Abruzzi and from Southern Yugoslavia to Ireland. The colour illustrations are from the excellent paintings of Marjorie Blamey. The geographical area covered is about the same as in that most successful book of 1967 by Anthony Huxley *Mountain Flowers* (Blandford: Poole, UK); but the lower altitudinal limit of 1000 metres adopted as a criterion for inclusion, has increased the

number of species illustrated, though most of the additions are really not "alpine". Over the wide range in latitude – from 42° to 71° N –spanned by this book the tree limit descends from some 2300 metres on the Alps to near sea level in Northern Scandinavia. Tree limit is usually taken as the lower boundary of alpine vegetation. It is ecologically much more significant than an arbitrarily fixed altitude, meaningless over such a huge latitudinal span. This is no criticism of the content, only of the title of this book. Anyhow, many of the readers will botanise from foothills to high mountains; for them this field guide caters well, in spite of its somewhat disconcerting title.

There are a number of good features. Text and illustrations are face to face. The

description of each species is appropriate and succinct, and it helps to have the diagnostic features in italics. Two keys based on drawings of flower shapes and one, for trees, based on leaf shapes help with preliminary identification. A number of plates give comparative drawings of one or other important diagnostic character in the case of the species of a few difficult genera or families: for instance, calices for the genus *Dianthus*, fruits for some umbellifers, and so on. The illustrated glossary is very clear.

Three minor criticisms: the use of English names for families; the invention of an English name, by translation from the latin binomial, for species which do not have one; and the omission of the author's

name after the botanical name. We should not fall into the deplorable habits of American field guides. This guide is dedicated to the Alpine Garden Society which celebrates its Golden Jubilee this year. It will make a most useful companion, as a pocket field flora, to the book *Mountain Flower Holidays in Europe*, just published by that Society. I am sure all members, and those of the sister Scottish Rock Garden Club, will need, and make good use of this guide.

G. Pontecorvo

G. Pontecorvo retired in 1975 as a member of the research staff of the Imperial Cancer Research Fund in London, UK.

Orchid growing

Orchids in Colour. By B. and W. Rittershausen. Pp.192. (Blandford: Poole, Dorset, UK, 1979.) £4.95.

THE attraction of this book by two well-known orchid-growers lies principally in Robin Fletcher's beautiful photographs, the authorship of which, despite the book's title, is not even acknowledged on the jacket. Over one hundred different species or hybrids are illustrated, sufficient to give an idea of the astonishing range of form and colour of these plants, whose increasing popularity is easily understood.

The introductory text comprises general remarks on the Orchidaceae, followed by separate sections dealing with the history and cultivation of the principal groups now grown. This first part suffers from an occasional tendency to become romantic at the expense of botanical accuracy, with such remarks as those with regard to nourishment (which "the orchid has learnt largely to do without"); the "meagre needs" of epiphytes "are totally satisfied by the moisture of the atmosphere". Fortunately in the separate sections that follow, quite full notes on various aspects of orchid culture are given, particularly as regards temperature and humidity (both of which are critical to successful orchid growing), the use of composts, and the timing of repotting and other cultural practices. Under modern centrally heated conditions, many orchids can be grown in a normal living room, and the suitability of various groups is duly noted. Brief notes on pests and diseases are also included in these sections of the book.

Apart from the breeding of new hybrids, large numbers of which are created every season, modern orchid propagation relies for many groups on "meristemming", the excision and subsequent culture of the growing tip of a shoot; and it would have been useful to include a more detailed description of this practice. There is also virtually no account of the genetics of the

extremely interesting and complex nature of multigeneric hybridisation.

Detailed notes (facing each plate) are given on the history and culture of the actual varieties illustrated, and these also deal with related species or hybrids. As a whole, however, the text could do with considerable editing: besides a tendency to be repetitious and thus sometimes confusing, it is at times so ungrammatical as to be obscure and irritating for the

reader to a degree that should not occur in a book of this style and authority. One point in its favour is the small format of a volume that one might have expected to see published as a much larger 'coffee-table' book.

Peter Collins

Peter Collins is a freelance science writer who has been studying the orchids of Europe for more than 25 years.

Man's use of dry lands

Dry Lands: Man and Plants. By R. and M. Adams and A. A. Willens. Pp.152. (Architectural Press: London, 1979.) £12.95.

This volume is written by two landscape architects, a horticulturalist and an agriculturalist, who are concerned to put their expertise to use in the careful management of dryland ecosystems. As a background to this aim the categories of arid land and their special characteristics are represented in some detail. Causes of aridity are discussed, and methods of supplying an index of its severity presented. This is followed by a survey of Northern and Southern Hemisphere desert zones; desert morphology and soils are presented next and desert vegetation types described, including methods of adaptation by plants to arid conditions. This leads on naturally to plant selection for arid regions and the way in which man may manage and modify desert ecosystems. Subsequently, survey and planning methods are expanded, and design and implementation procedures are outlined.

To deal in detail with such an extensive remit would be difficult and in seeking ways to overcome these difficulties the authors have been both inventive and shallow in turn. Overall, the book is attractive in both its presentation and treatment; it follows through from ecological considerations to methods of developing areas agriculturally, for forestry and horticultural

purposes, and then to urban landscaping and the development of a coastal site — marina, shops, hotels, airport hotels and gardens. The designs for urban spaces look attractive but the nearest I have experienced to them, in practice, is in Venezuela rather than in the arid zones of Africa or the Middle East. In the course of developing such plans a site survey checklist is presented. This includes hydrological features, geology, topography, soil characteristics, climate, and ecological components. Enough detail is given of the necessity to consider each feature in turn but usually this would not allow a basic understanding of the way in which the various factors operate or how they interact. Where attempts are made to give a basic understanding (as where features of C₃, C₄ and CAM photosynthesis are explained) these can be confused in their presentation and attribute findings to authors who have merely carried along investigations initiated decades previously by other workers.

As an interesting excursion into the application of ecological expertise to planning and land management in difficult situations, this book can be recommended. The work is a useful guide for the ecologist seeking to apply his knowledge. For the planner or landscape architect, seeking the scientific basis for his art as applied to the arid zone, it falls short of a *vade-mecum*.

M.J. Chadwick

M.J. Chadwick is Senior Lecturer in Ecology at the University of York, UK.

Fascination for fossils

FIVE recently published books on various aspects of prehistoric life and the evolution of the vertebrates are reviewed here. All are intended to help the layman realise the fascination that fossil life might hold for him.

The Hamlyn Nature Guide to Fossils (Hamlyn: London; £1.95), by Richard Moody, begins with a brief introductory section covering geological time, fossilisation and fossil collection, preparation and curation. There is then a simple key to the identification of fossil material down to phylum or class level. The bulk of the book consists of attractively presented colour photographs of some 200 fossils, mostly identified to generic level accompanied by a description and a brief statement of range, both stratigraphical and geographical. Most of the fossils are of common occurrence, though one or two are to be considered rarities; many have world-wide distribution.

The generic identifications are virtually all correct, and the balance of different groups is nicely judged. The various invertebrate fossil groups are treated in turn, followed by a few vertebrates, trace fossils (strangely conodonts and *Serpula* appear under this latter heading), and plants.

My main criticism concerns the photographs. Several are larger than the frame size allows so that parts of the fossil are missing beyond the margins of the plate. Others are presented in rather unconventional orientations. In other cases more than one view is needed and this could have been easily achieved within the frame-size, as the example of *Leptaena* shows. However, apart from a few ill-preserved specimens and a handful of poorly lit ones, the photographs are of high standard and there is never any doubt about what is depicted.

Although there are labelled diagrams to illustrate morphology, I felt sometimes that the descriptions became a little too technical for the amateur fossil collector, to whom the book is directed. He will no doubt welcome it as an attractive and useful addition to his library. The book would fit into a good-sized pocket and could be used in the field.

Fossils (Collins: London; £3.50), by David Dineley, aims to fill a gap which exists in the palaeontological literature; it is thus neither a palaeontology textbook, nor a field guide to fossil identification. The author has produced a book which presents, in a very readable way, the subject of palaeontology to the layman.

After brief introductory sections on sedimentary processes, geological time and evolution of life, there follows a chapter on plants which, in an effort to be comprehensive, perhaps errs too much towards the rarely fossilised non-vascular plants. Treatment of animal fossils follows

mode of life, with plankton and nekton being described before benthos. Terrestrial life then follows, leading on naturally to vertebrates and vertebrate evolution.

Having thus covered the major groups of organisms, the author then looks briefly at fossil communities. Techniques of fossil collecting are prefaced by a code for fieldwork and several conservation-minded caveats. The concluding section suggests areas in Britain where fossils may be sought, with further reading for each area discussed. There is a useful list of books for those interested in going into the subject more deeply.

The book is illustrated with numerous line drawings and sixteen black-and-white plates. Two of the latter were badly blurred in my review copy; I suspect this may be a unique fault to my copy but would-be purchasers should perhaps check the plates before buying. There are minor errors in some plate captions.

Professor Dineley's approach, together with his easy-to-read chatty style should win him applause from those who have been (or are about to be) seduced by the fascination of fossils.

Prehistoric Animals and Plants (Hamlyn: London; £2.95), though nominally by Josef Benes, must be considered essentially as a means of displaying reproductions of prehistoric life as seen by Zdenek Burian. After an introductory five pages of text on the origins of life and on invertebrates, there follows some three dozen more pages of text devoted almost exclusively to vertebrates.

After this preamble we are into the bulk of the book, which consists of coloured pictures of animals or faunas as they may have appeared, accompanied by a descriptive text which has been well translated from the original Czechoslovakian. The plates are reproductions of original paintings by Zdenek Burian and are a tribute to his skill in convincingly reconstructing the animals in their environments.

Although this book is up to date in some respects (it contains, for instance a Precambrian reconstruction based on the Ediacara fauna), in other cases it suffers from being behind the times — as for instance in the pictures of dinosaurs which are based on the "giant lizard" concept of dinosaur physiology. *Brachiosaurus*, for example, is relegated to a watery life, as it has been so often in the past. It is to be regretted that the book is so heavily vertebrate oriented particularly because the few reconstructions of invertebrates are so successful.

The standard of the pictures varies, but it is not clear whether this is entirely due to faulty reproduction; some colours in a few pictures are rather garish, a few images appear to be out of focus, and some pictures were originally painted on a rather coarse canvas to the detriment, sometimes, of detail in the reproduction. The work

must represent the output of the artist over a considerable period so that differences in style are not unexpected.

It is not clear to whom this book is directed, but at the remarkable price of £2.95 for a hardback book with over 300 pages and more than 130 colour pictures, it should find a ready market in anyone interested in realistic reconstructions of extinct vertebrates.

The Evolution of the Mammals (Peter Lowe: London; £4.50), by L.B. Halstead, begins with a definition of what constitutes a mammal, and then explores briefly and succinctly geological time, evolution and the fossil record, the origin of life, and then goes into more detail on vertebrate evolution. Thus by the time that mammals are first discussed in detail, the reader should be fully aware of their relationship to other vertebrate groups and their range through geological time.

The Mesozoic mammals are dealt with rather sketchily, but there is a fuller treatment of Cainozoic mammals, initially system by system, in which differences between successive faunas are spelt out clearly in the text and by superb coloured illustrations; some of these illustrate different genera of particular mammalian groups and others, reconstructions of mammalian faunas of different ages in their supposed environments. The pictures are very successfully executed and the considerable impact of this large-format book owes much to them.

There are sections on the evolution of man, on the different faunas to be found in the main continental areas and even predictions on what the future holds for the mammalian class.

The whole book is clearly written, interestingly presented, and succeeds in telling the history of mammals through geological time in a most absorbing way. Although the key words for the British Library Catalogue are listed as "Mammals — Evolution — Juvenile literature", I venture to suggest that most parents who buy this book for their offspring will find it fascinating reading for themselves.

The subtitle of *Archosauria*, by J.C. McLoughlin (Allen Lane: London; £4.50) — *A New Look at the Old Dinosaur* — also appears in large print on the dust cover and will no doubt cause confusion with Alan Charig's new book *A New Look at the Dinosaurs* (Heinemann: London, 1979). However, in content John McLoughlin's book is much closer to Adrian Desmond's *The Hot-Blooded Dinosaurs* (Blond and Briggs: London, 1975).

A zoologist by profession (as are many vertebrate palaeontologists), the author is entirely sold on the idea that dinosaurs were endothermic (warm-blooded) animals, and the book considers the Archosaurs as a class of vertebrates (following the line first proposed by Bakker and Galton).

Each group of archosaurs is discussed and their functional morphology in the

light of an endothermic physiology re-interpreted. The text is well written, though the British publishers have apparently reprinted the American edition, as American spellings persist. The author has provided his own illustrations and succeeded remarkably in selling his argument by managing to give an intelligent and alert expression to the faces of the long-thought slothful dinosaurs. The arguments are put over clearly and persuasively, but the author too rarely considers alternative viewpoints to his own. I found his attitude to classical

dinosaur reconstructions somewhat patronising, and felt that the criticisms of earlier workers were not entirely justified. Despite his biased view, the author has produced a book which is certainly stimulating, very readable and attractively presented, and which will appeal to anyone with an interest in dinosaurs, whatever their background.

John C.W. Cope

John C.W. Cope is Senior Lecturer in Geology at University College, Swansea, UK.

In the hands of the rockhounds

MINERALOGY is increasingly in the hands of the rockhounds. Short courses in gemology and the lapidary arts are over-subscribed and will hopefully lead on to a much wider public interest in the earth sciences in general. Sadly, one consequence is that well-known mineral localities, and some which once were near-secret, are picked over and almost barren. Disused workings are being closed for safety reasons and recent legislation all but bans collectors from active mines. For all except the mundane, many collectors are forced to buy imported specimens and professional collecting has become highly profitable. It is not unknown for *bona fide* scientific collectors or the casual amateur to be literally driven off outcrops by well equipped and frenzied dealers or their suppliers. In this context, the publication of three new books aimed at the amateur can be received with some misgivings. Not one of them pays any attention to the pressure on sites nor to conservation practices. That being said, professional geologists should attempt to encourage wider interest and activity in this field as a route to popularising earth sciences. But to what extent do the new books contribute to this aim?

The Hamlyn Nature Guide to Minerals by Andrew Clark (Hamlyn: London and New York; £1.95) is a thin book, in all respects, it claims to be a practical identification guide but its brief introduction to mineral properties is too dry for the beginner. Its 436 colour photographs are pretty enough, but the brief mineral descriptions are not arranged in a suitable format for easy identification. The order is the conventional composition/atomic structure classification, which is no guide to minerals' immediate appearance. No information about localities is given but there are very brief notes on geological setting, which will certainly not aid the non-geologist to find

good specimens. Perhaps this is a book for the pure conservationist.

The Audubon Society Field Guide to North American Rocks and Minerals by Charles W. Chesterman (Knopf; New York; \$9.95) is in another class. It contains 794 magnificent colour photographs and is virtually a self-contained introduction to geology through its more aesthetic products. The book begins with a simple but comprehensive account of the diagnostic properties of minerals. The ordering of photographs does not match that in the identification tables or the extended mineral by mineral descriptions — the photographs being arranged by colour and form, the tables by colour and hardness. But this is only a minor irritation, albeit unnecessary. The quality of specimens was a little daunting for this casual collector and perhaps for even more determined rockhounds. This reflects the use of tiny specimens or micromounts — forced on

modern collectors by the self-induced dearth of 'mantle piece' specimens. Common rocks are well treated and sufficient space has been devoted to introducing the elements of the science, as well as the hobby, to make this book a notable contribution in the field and excellent value.

Rock and Mineral Collecting in Britain by Peter R. Rodgers (Faber and Faber: London, hardback £6.95, paperback £3.50) is a good text for absolute beginners and relates mineral occurrences to geological setting quite adequately. However, the simplification into igneous, sedimentary and metamorphic classes of deposits can be misleading at this elementary level, and arguably a better approach would have been regional descriptions of famous mineral sites. The localities listed seems impressive, but the reader soon finds those mentioned in the text very elusive. The most useful items are the mineral catalogue and the list of localities with grid references. There are glaring omissions among the localities and the list would have been better organised region by region with accompanying maps. Several famous British minerals, notably mimetite, harmotome, torbanite and alstonite are inexplicably absent. The beginner will find this an encouraging little volume, but a little extra documentation might have found a ready demand among more experienced collectors.

Stephen A. Drury

Stephen A. Drury is Lecturer in the Department of Earth Sciences at the Open University, Milton Keynes, UK.

How to grow your own rubies

If you want an excuse not to give your wife an opal for Christmas, you will find one in D. Elwell's book: *Man-Made Gemstones* (Ellis: Chichester, UK; Wiley & Halstead: New York; £15), both natural and synthetic opals are porous, and serious cracking can occur if they are immersed in warm soapy water.

Far better to give her a ruby — "Growing rubies by the flame fusion process in the garage is possible, but not recommended. We will therefore consider flux growth" . . . Dr Elwell goes on to give detailed instructions, but briefly you take a platinum crucible, mix lead fluoride with the oxides of lead, boron, aluminium and chromium, add a pinch of lanthanum oxide to improve crystal shape, put in the oven at 250°C for 24 hours, allow to cool slowly, and extract your crystals using nitric acid.

Unfortunately, your home-grown rubies, though real, will be "synthetic". This is an important qualification.

Synthetic gemstones are identical, chemically and crystallographically speaking, to the real thing; but they are usually distinguishable from, and therefore less valuable than, natural gemstones. 'Imitation' or 'simulated' gems on the other hand resemble the real thing but are actually a different material; thus yttrium aluminium garnet (YAG) and cubic zirconia are both used as diamond simulants.

Opals do have their own fascination, as Elwell explains, even if you cannot yet grow your own. They are unique among gemstones in being an ordered array of silica crystallites, whose spacing leads to diffraction of visible light and hence to a captivating play of colours as the gem is moved. Most other gemstones are genuine crystals — an ordered array of atoms — and are transparent, whereas opal is opaque.

I certainly enjoyed reading Dr Elwell's book. Although ruby is the only crystal he gives a recipe for, he also describes the synthesis of sapphire, spinel, emerald, diamond and its simulants, and many other precious and semiprecious stones.

Gemology (Wiley-Interscience: New York and Chichester, UK; £13.50) by the

Professor Emeritus of Mineralogy at Harvard (C.S. Hurlbut) and the Curator Emeritus of Mineralogy at the Smithsonian Institution (G.S. Switzer) is a self-confessed "textbook" aimed at gemology courses as well as at jewellers and collectors. As such it is less readable than *Man-Made Gemstones*; but it is much better as a work of reference — as well as being bigger (in number of pages and page size) and cheaper.

The book covers origin and occurrence of gemstones, crystal chemistry and crystallography, physical and optical properties of gems, gemological instruments, synthesis of gems, imitation gems, cutting and polishing. The longest chapter, "Descriptive Gemology", tabulates all the important features and

characteristics of the common gemstones (and some uncommon ones) with particular emphasis on how to tell whether the gem is natural, synthetic or imitation. There are also twelve full-page colour plates of superb quality.

These books are complementary, rather than being in direct competition; and both would make suitable presents for your favourite lapidary or jeweller, or for anyone with a scientific interest in gems and crystals. But if you can afford only one of them, *Gemology* is the better buy.

John Walker

John Walker was a Research Fellow at the Universities of Reading and Paris, studying the optical properties of diamond. He is currently a microprocessor consultant.

Everything you wanted to know about cancer but were afraid to ask

DISEASE has always haunted the human imagination. Leprosy, the black Death and other horrors have in turn stalked the misty frontiers of the collective unconscious. As the lethal epidemic diseases recede into history the archetypal phobias of Western Society are shifting their attention to cancer. One of civilisation's most awful self-inflicted wounds, cancer has become, like death itself, a taboo. In the third world the peasants flee to the hills when cholera strikes; in our affluent society there is nowhere to run to avoid what the late John Wayne called the big C. In the belief that fear can be abolished by knowledge (or rather, information) two recent books have been launched — from different directions and aimed at different audiences.

The Cancer Reference Book (Paddington: New York and London; £4.95), by P.M. Levitt and E.S. Gurabrick, is written by two American Professors of English using information provided by a small team of established cancer specialists. It is deliberately written in simple English, avoiding all technical language or complicated concepts. The style is light with quotations from Dylan Thomas, Robert Lowell *et al*, and it is rigorously honest. There is no obfuscation, no undue optimism. If a

treatment does not work, the book says so. Reassurance is offered only when justified. It provides brief descriptions of the various forms of cancer and provides lists of questions and answers. The questions are real — the sort of questions asked by patients and their relatives. The information provided is strictly limited to topics of immediate relevance to patients with malignant disease and to the uninformed general public. Symptoms, treatments, prognosis and terminal care are well covered. The long section on widespread cancer and terminal care is very well presented and pays an envious tribute to the British hospice movement. Some aspects of the dialectic are (at present) peculiar to North America. Unproven treatment methods such as laetrile and the orgone box receive a lot of critical attention and furthermore the authors recommend that all men over 50 should have a rectal examination once a year. They also deal with "cancer insurance" in some detail, as in the USA 2 million such policies are sold every year. The cost of treatment for breast cancer can be as high as \$100,000 per patient.

Assuming that the reader knows absolutely nothing, the book contains simple line drawings most of which are probably helpful. However, one picture of the mouth labelled "the mouth" seemed just a little superfluous. However, this volume cannot really be judged by doctors. It is for the general public and for patients. To assess its value I have just completed a small uncontrolled trial by inviting questions from patients, relatives and friends. Each and every question was answered, and answered well, although finding them by referring to the index was a little frustrating at times. *The Cancer Reference Book* contains, as advertised in its subtitle "Direct and clear answers to everyone's questions". It must therefore be judged a considerable success. Its honesty will I suspect upset some doctors more than their patients. It could make life a little difficult for those, who, with

euphemisms and woolly reassurance, use deception as a form of therapy. *The Cancer Reference Book* could be a blow for patient's lib.

Cancer: the Outlaw Cell is an edited collection, by R.E. La Fond, of articles originally published in *Chemistry* and now dressed up and issued by the American Chemical Society (Washington DC; \$15). It is a distinctly upmarket product. Written originally for chemists (no, not pharmacists) it now seems to be addressed to what I can only describe as "a BBC2 audience". Expensively produced and beautifully illustrated its approach is conventional but slick (witness the title). Clinically it is uninformative and contains little or nothing about what cancer is or what it does to people. It is essentially a basic primer in some current trends in cancer research, very basic in places. An introduction to cell chemistry, for example, takes less than a page and a half. There are, however, some excellent general essays. Dr Armin C. Braun's chapter on cancer as a problem in development will fascinate anyone with an O level in biology. There are also comprehensive chapters on chemical carcinogens, oncogenic viruses, the cell surface and the inevitable immunology.

A simple but concise essay on cancer chemotherapy by the Drs Burchenal highlights a central issue in contemporary cancer research. The logical approach to finding new drugs, they say, would be to find differences between normal and malignant cells and then exploit the difference. However, as this approach has been tried in the past and failed, it has been replaced almost totally by empirical drug screening, looking for anti-proliferative agents, agents which by definition, are bound to be toxic. To illustrate the current obsession with "multi-modality therapy" they provide an unfortunate analogy. The body, they say, is like a lawn, normal cells are grass and cancer cells are weeds. Surgery is therefore the equivalent of hand weeding, radiotherapy is a flamethrower and chemotherapy is (or should be) a selective weedkiller. Stimulation of the immune response is the equivalent of stimulating the grass to choke the weeds. As they put it so succinctly, immunotherapy is fertiliser.

It is an optimistic book, the authors of each chapter arguing strongly for their own line of research and its potential value. It is an excellent bit of public relations for American cancer research; no room here for self-doubt, for the currently fashionable pessimism. As an introduction to cancer for the scientifically literate layman it could make a fascinating read.

G.A. Currie

G.A. Currie is Reader in Tumour Immunology at the Institute of Cancer Research, and Honorary Consultant Physician at the Royal Marsden Hospital, Sutton, UK.

● In the review of *Malignant Neglect* (see *Nature*, 282, 166; 1979), the first sentence of the second paragraph should have read: "A normal metabolite, ammonia, is called (page 105) a "toxic organic vapor"."

Man's place in Nature

The Ancient Science of Geomancy: Man in Harmony with the Earth. By Nigel Pennick. Pp. 180. (Thames and Hudson: New York and London, 1979.) \$16.95; £5.95.

FOR those who believe that a proper understanding of our world can come only through the application of the scientific method — by observation, hypothesis, prediction and test, leading to verification or otherwise — this book will read as mystical nonsense. For those who believe that there are other, less rigorous ways to the truth, who prefer a mystical to an archaeological or historical approach to the past and who are also attracted to the idea of a return to a technologically simple and harmonious human culture, it may well have considerable appeal. This conclusion is quickly reached by reading the Introduction in which the author's basic assumptions are clearly stated they are vast, irrational and of a nature that precludes almost any kind of constructive criticism.

"The practice of geomancy, which may roughly be defined as the science of putting human habitats and activities into harmony with the visible and invisible world around us, was at one time universal, and vestiges of it remain in the landscape, architecture, ritual and folklore of almost all countries in the world. This remarkable series of correspondences between different cultures has been held to be evidence for a former world civilisation . . . These archetypal patterns . . . have produced the worldwide occurrence of outward form and inner purpose found in geomancy. Hence the seeking of cosmic power points on the surface of the Earth, special places where the mind can expand into new levels of consciousness . . ."

If one accepts these basic assumptions — a better phrase would be "this basic faith" — then all the rest follows. The book elaborates on the theme, describing a wide range of curious facts and phenomena as if their mere recounting is enough to prove the author's case. The Thoms' work on standing stones is inevitably brought in, as if this particular example of the refutation of the old simplistic idea of the steady progress of man from savagery to modern urban civilisation also proved the existence of a world-wide science in antiquity. Ley lines appear, as does dowsing; and holy wells, holy hills and geometrically designed buildings are thought of as places where the natural energies of the Earth were identified and tapped in ancient times. Proofs of the power of these energies — as of all aspects of geomancy — are invariably sought in unattributed and unverifiable reports of "evidence", as of an allegedly sacrificed animal removed from its burial place, of disasters following and ceasing with the return of the corpse to its tomb. Adverse criticism is exorcised in advance by allegations of bias and prejudice among

professional scientists and scholars. The mind reels at the task — fortunately unnecessary — of analysing and assessing this mass of diverse and incoherent material. Probably it is best simply to regard the work as a compendium of bizarre and interesting phenomena and tales.

One general point, however, is worth making. The thread running through the book is that in early times (what archaeologists call the Palaeolithic, or hunting and food-gathering period) man lived in complete harmony with nature and that this harmony was later disrupted with the invention of agriculture. In a typical phrase, full of apparent meaning but defying close analysis, "settled life necessitated the enclosure of space, a stopping of the free flow of the spirit of the earth . . .". All signs of ancient skills and wisdom are interpreted in terms of early Man's knowledge and utilisation of the Earth forces assumed to exist behind such harmony. However, this "harmony" can also be interpreted quite unmythically in terms of the natural (that is, unconscious) selection of technologically primitive societies so that those survived which were best adapted to their environments. No alien spacemen, antediluvian Atlantean

civilisations or practitioners of a world-wide geomantic science are needed — but a thorough knowledge of the available archaeological and historical evidence is.

This makes the point forcibly that, in the 120 years which have elapsed since the publication of *The Origin of Species*, the application of the theories of evolution and natural selection to the development of man and his societies has made too little headway. The second half of the Darwinian revolution is thus still incomplete and, as a result, a host of beliefs and dogmas based on assumptions about the special nature of *Homo sapiens*, and on his independences, of natural laws, continues to flourish. Until, in T.H. Huxley's phrase, "Man's place in nature" is properly and widely understood and accepted there will still be massive markets, even among educated people, for books of this kind in which the effects of natural selection are viewed anthropocentrically and attributed to primeval wisdom, or are explained by supernatural or extraterrestrial agencies.

Euan W. MacKie

Euan W. MacKie is at the Hunterian Museum, University of Glasgow, UK.

Glimpses of volcanoes

Volcanoes. By M.B. Lambert. Pp.64. (David and Charles: London and Newton Abbot, UK; University of Washington Press: Seattle, 1978.) £3.95; \$10.95.

It is amazing how little the author has managed to cram into this slim volume. The book runs to 64 pages, over half of which are devoted to pictures and diagrams, and it is divided into 10 principal sections, each of which consists of a few pages of text, some relevant pictures, a surprising amount of blank space and a vaguely volcanic snatch of verse. The sections cover fairly conventional topics such as "Major volcanic landforms", "Products of explosion", "Volcanoes in time and space", and so on. Unfortunately, the contents of the sections do not match up to their worthy titles. The section on "Volcanoes in time and space", for example, runs to only a few hundred words, about half of which are devoted to Canada.

It is difficult to see quite what kind of a book the author was aiming at. One could make a strong case for the publication of a good coffee-table book on volcanoes, but this one does not come anywhere near it, as although many of the photographs used are good, they are not numerous enough,

not glossy enough, and not in colour. The book is also emphatically *not* a "simply yet thorough introduction to volcanoes", as the dust-jacket claims, but is instead a patchy, misleading digest of information on a subject which is inherently very popular among lay-readers.

Such readers are likely to fare badly from Lambert's book. What, for example, is the uninformed reader going to make of the 400 words on page 9, which includes a discussion of thermal gradients, upper mantle composition, partial melting and fractional crystallization? Not much, I suspect. The best that can be said about the text is that it is at least accurate for the most part, being derived from respectable sources to whom Lambert makes due acknowledgement. In many cases, however, Lambert's use of these sources is curiously ill considered. He quotes, for example, Macdonald's figure of 1,800,000 km² for the extent of the so-called 'Thuelan Plateau'. It is now generally accepted that it was never anything like this size, the various components having been widely separated by seafloor spreading. Macdonald even goes so far as to suggest this possibility, but Lambert ignores it.

Peter Francis

Peter Francis is a Lecturer in Earth Sciences at the Open University, Milton Keynes, UK.

Frozen Earth

The World of Ice: A Natural History of the Frozen Regions. Pp. 120. By B.S. John. (Orbis: London, 1979.) £5.95.

THIS attractively produced book would look well on any coffee-table and should arouse interest in the World's ice in all its varied manifestations. Brian John has written a stimulating and, for the most part, basically sound account of the ice-covered or frozen regions of the Earth that assumes little acquaintance on the part of the reader with technical terms, whether geographical, glaciological or biological. (A glossary is included.) It is therefore a book for the interested layman, the general reader, and only superficially for the student, the scientist or the naturalist. It is illustrated by about 100 superb colour plates, some covering a page or double-page spread; the layout is spacious, the quality of production high and the price very reasonable.

The text is clearly written but, inevitably, compressed and simplified, so that the unwary or a reader unfamiliar with the subject can be misled at times. Just to select a few examples, we are told that the World's climate has for most of the time been very much colder than at present (page 10), ignoring the long spans of the geological past when it has actually been much warmer, with no polar ice; that the strandflat was "eroded by the sea" (page 19); and that a mechanism of great importance to the movement of the Antarctic ice-sheet is basal sliding (page

47), giving the unfortunate impression that it is peculiar to the Antarctic. The caption to a photo of northern Skye (page 32) describes it as "glacial terrain" but the main processes actually depicted are connected with mass movement; sheet-jointing in Yosemite (page 61) is attributed to temperature variations (no mention of pressure release); and the caption to a photo of Bodmin tors (page 60) gives the impression that frost was the major formative process. In all such statements there may well be a grain of truth, but brevity and sometimes an unfortunate choice of words can easily convey quite the wrong idea. The opening page (page 7) is distinctly journalistic ("We are all basically afraid of extreme cold and darkness"); and the inhabitants of the Alps or Scandinavia must be amused to read that "Something akin to panic is induced... every time there is a heavy snowfall".

There are seven chapters: the ice planet; ice environments; glaciers; frozen ground; ice afloat; plants, animals and ice; and Man in the World of ice. Some important subjects are given little mention: avalanches are dismissed in about 70 words; the section on plants is comparatively thin; and in the chapter on Man, there is virtually no mention of the economic importance and utilisation of the permafrost areas of Siberia, Alaska and Arctic Canada. But the overall impression of the book is undoubtedly attractive and it should find a ready market on the bookshelves.

C. Embleton

C. Embleton is Reader in Geography at King's College, University of London, UK.

Immediacy and surprise

ASTRONOMERS are extremely fortunate members of the scientific community because they have an efficient public relations network. It may be an unwitting PR network — astronomers don't pay for it anyway — but the media do seem to be on their side. Two things are greatly in the subject's favour. First is the immediacy. The results and images from a spaceprobe passing Jupiter, for example, are transmitted and processed with great speed. The time lag between observation and announcement is short. Second is the element of surprise. Time and again the astronomers find things they did not expect. Jupiter and its satellites again furnish two examples. It would have been a brave astronomer who firmly predicted the existence of active volcanoes on Io or a ring around the planet. Pioneer found both. New things seem to crop up wherever the astronomer looks and nothing seemingly interests the media more than novelty

coupled with those looks of amazement on the faces of scientists when confronted with something unexpected. Two consequences result: the number of people interested in the subject continues to increase, and astronomy books seem to date quickly, providing ample room and justification for new works and editions.

This year, as in previous years, I am confronted with a large pile of new books and I am again trying to answer the question "Which book would you, young astronomer, old astronomer, amateur or professional, like to find in your stocking on Christmas morning?" The choice is enormous and the answer is far from easy. One which I am sure will go down very well is *The Guinness Book of Astronomy Facts and Feats* (Guinness Superlatives: Enfield, UK; £6.95), compiled by Patrick Moore. It is the quiz fanatics' dream, a fund of answers to questions you probably would not have asked yourself in the first place. Every dip I made into the book produced something of interest and humour. Who would have thought that Le Monnier observed Uranus eight times in four weeks and failed to identify it as a planet, or that

one of his observations was later found scrawled on a paper bag which had once contained hair perfume; that the longest living sunspot group lasted for 200 days; that Halley's comet was excommunicated in 1456; that the smallest known galaxy is only 1,000 light years across; that a piece of the Barwell meteorite was found nestling in a vase of artificial flowers on the windowsill of a house in the village? The book is full of them; you can imagine the advertisement, "astound your friends", "impress your colleagues" — but it's all good fun and excellent value. The facts and feats are listed in sections, a random few being Sun, Moon, Jupiter, double stars and galaxies, so they are easily accessible. Eighty-seven pages are taken up by a star catalogue which struck me as being somewhat out of place. The star maps in this catalogue were too small, had no right ascension or declination grid and the bright stars in each constellation were joined in a most unusual, idiosyncratic fashion. And I am sure that 10^{-8} cm as the definition of an Angstrom unit is easier to comprehend than "one hundred millionth part of a centimetre." Anyway, these are all minor quibbles. I know few astronomers who would not be delighted to receive a copy.

The same can be said of the next book, *Space Art* compiled and written by Ron Miller (Starlog Magazine, O'Quinn Studios: New York; paperback \$7.95, slipcase deluxe \$12). We are treated to a multitude of colour plates of astronomical and space science subjects realised by a host of artists. The book continually underlines the usefulness of space art. These paintings synthesise scientific knowledge and data into a visual and concrete form. Flesh is put on to the bones of numbers, equations and theoretical predictions. Whenever a new phenomenon or discovery comes to light it is the space artist who asks the question, "What would it actually look like to the human eye if you could stand there and see it?" Ron Miller traces the evolution of this art-form from its infancy in 1865, when *From the Earth to the Moon* and *A Trip Around the Moon* by Jules Verne, contained illustrations created by the artist painting for the first time strictly according to scientific fact, through to the present day, where we benefit greatly from such organisations as the NASA Fine Arts Programme. NASA decided that, despite filmed documentation of all their projects, art would give their work a special emotional impact and significance. Miller goes as far as to propose that it was the space art in the popular illustrated magazines and books of the early 1950s that helped rally the taxpayers behind the space programme. Well of course, this is debatable, but the worth of the present book is beyond dispute. It is excellent value for money, a continual feast for the eye and the imagination and an ideal gift.

Let us now turn to two books for the observer. The Mittons, Jacqueline and Simon, have produced a *Star Atlas*



(Jonathan Cape: London; Crown: New York; £1.10). This is a very handy, reasonably priced, 32-page, large-format paperback, half of which is devoted to a description of such topics as mapping the skies, the constellations, star names, magnitudes and the way the atlas is to be used; the remainder contains the maps, 2 for the north and south celestial poles, declinations from 90° to 60°, and 12 for the equatorial region of the celestial sphere, each of these being 5 hr across, allowing some overlap. My first response to this book was to compare it with Norton's *Star Atlas* (Gall and Inglis: Edinburgh; £4.50). Norton's book goes down to magnitude 6.35 and contains slightly more stars than the Mittons'. Norton represents his stars by filled black dots, scaled in half-magnitude steps. The Mittons represent stars by spidery, computer-drawn asterisks, the star magnitudes being indicated to within 0.1 mag by the size of the printed star image (you need a good engraved scale and a magnifying glass to make use of this).

There is considerably more text in Norton than in Mitton, although people who can get most from the atlas do not really need the text. There is a more comprehensive list of doubles, variables, clusters and nebulae in Norton. I prefer Norton's dots to the Mittons' asterisks, although I prefer the Mittons' price to Norton's. However, if I was buying an amateur star atlas I would still save up for Norton.

The second book for the amateur observer is *The Messier Album* by J.H. Mallas and E.Kreimer (Sky Publishing Corporation: Cambridge, Massachusetts; Cambridge University Press: Cambridge; \$9.50; £6.95) and I recommend this most heartily. Owen Gingerich provides an historical introduction to Messier and the catalogue is reproduced in facsimile, but the real kernel of the book is the descriptions, drawings and photographs of each object. We are not confronted here with 100-inch

Schmidt photographs but with the realistic capabilities of amateur astronomers. Mallas has a 4-inch refractor and he draws and describes exactly what can be seen of each Messier object using this instrument. The photographs were obtained by Kreimer using a 12.5-inch *f*/7 reflector, with the guiding head, low-temperature, plate camera at the newtonian focus. About eight pictures could be obtained during a 4-hour observing session. The book ends with a series of colour photographs and again the technique used to obtain each of these is given in detail. Everyone with a telescope should have this book.

The remainder of my Christmas books have a bias either towards the fundamentals of astronomy or towards space science. *Basic Astronomy* by Patrick Moore (Ian Henry: Hornchurch, UK; £3.95) is a modest, small-format second edition of the 1969 original. The production quality reminds me of the books published around the time of the second World War, most of the photographic reproductions being worse than one would see in the average newspaper. The figures suffer from *déjà vu*, all seemingly having been plucked from some ancient tome. The text is vintage Patrick Moore and I like it. You cannot help being infected by his enthusiasm. He writes about what he knows; you could

drive a cart and horses through the omissions. At the same extremely elementary level is *Discovering Astronomy* by Jacqueline and Simon Mitton, Institute of Astronomy, University of Cambridge (Longman: Harlow, UK; Prentice-Hall: Englewood Cliffs, New Jersey; £4.75). I quote the address of the authors in full because this is given in large letters on the cover. We are also told on the cover that "For the inquiring young person there is no first-step introduction to the 'new' astronomy written with the authority commanded by authors at Cambridge University's Institute of Astronomy." Why should we care where the authors come from as long as they have written a good book? I am sure that an author could write a 'first step' into astronomy without going anywhere near a university, never mind Cambridge. Inside we find a superbly illustrated 90-page introduction to astronomy, ideal for the infant and junior school room. Adults wishing to take more than one step into astronomy should start elsewhere. However, I would not specifically recommend *Foundations of Astronomy: From Big Bang to Black Holes* (David and Charles: Newton Abbot, UK; £5.95) by Richard Knox. It is billed as a new and radically different introduction to astronomy for the lay or student reader. I found it to be neither new nor radical but simply a reasonably competent,

Published today:

Zoogeography and Diversity in Plankton

Edited by S. van der Spoel and A. C. Pierrot-Bults

The proper management of fisheries and man's other oceanic activities depends on a detailed knowledge of marine primary and secondary producers. Whilst primary producers have been well covered in the literature, the secondary producers have been, until now, comparatively neglected. Drs. van der Spoel and Pierrot-Bults have set out to redress the balance by drawing together leading workers from seven countries to write this book on zooplankton.

Throughout, the authors make use of the most recent data and take account of the newer theories and methods that are emerging. Together, they have produced a comprehensive account of plankton zoogeography throughout the world that will serve as an authoritative reference for students and research workers in marine biology.

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Edward Arnold

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methodical plod through the usual topics.

As time is short and the number of simple astronomy books enormous, it is important that the new reader, young or old, does not waste time or become discouraged by making the wrong choice for his introductory text. The Americans come to our rescue here. I have previously praised *University Astronomy* (Saunders) by J.M. Pasachoff and M.L. Kutner, and *New Horizons in Astronomy* (Freeman) by J.C. Brandt and S.P. Maran, and I have no hesitation in adding the works of William J. Kaufmann III to this short list. He has produced a trilogy of books for the general reader. I have before me *Planets and Moons* and *Stars and Nebulae* (Freeman: San Francisco and London; hardback £8 and £8.20, paperback £3.95 and £4.10). The line drawings are superb, clear, uncluttered, eminently understandable and a perfect example to every writer of how it should be done. The illustrations are beautifully reproduced and relevant to the text. Not for Kaufmann the collecting together of any handful of NASA prints just to add spice. The text hammers home the important points, the new ideas, the problems and the next steps. It is unfair to single out one topic from the rest but I will. Kaufmann's exposition of the birth, life and death of stars is masterly. This is how introductory books should be written. There is no need to plod through mediocrity when the best is so easily available.

Saturn and Beyond (Lothrop, Lee and Shepard; New York; \$7.95) by Isaac Asimov, is a rather pedantic stroll through the features of Saturn, Uranus, Neptune and Pluto. We are told that this is Dr Asimov's 199th book. I got the impression that Dr Asimov has slightly run out of steam on this one. Why are rings common? How many theories of ring origin are there? Why do these planets have similar spin periods? Why haven't we detected planets beyond Pluto? Why were Neptune and Pluto so difficult to discover? Why are the spin axes at different inclinations, and why are the interior structures of these planets dissimilar? We learn too little about these problems in this book; we are treated to too many tables, too little explanation, too few illustrations. Even the US Naval Observatory's photograph of Pluto and Charon, arguably one of the most exciting outer planetary photographs of the decade, is missing. Never mind, Kaufmann's book has it.

The Astronomy Data Book by J. Hedley Robinson and James Muir (David and Charles: Newton Abbot, UK; £6.95) is a revised but little improved version of the 1972 original. I must not be too harsh with this book because it contains many useful tables listing astronomical objects and their characteristics. It is also aimed much more towards the amateur astronomer than C.W. Allen's classic *Astrophysical Quantities* (Athlone Press: University of London). However, I cannot help but feel

that it could be made much better. The Glossary is very poor, and throughout the book the definitions need tightening up, with more quantitative assertions as opposed to qualitative ones. The few figures the book contains leave much to be desired and many more figures are needed. The knots in which the authors tie themselves as they try to describe the Moon's orbit, collars on Venus, zodiacal light and sidereal time, to mention but a few, have to be read to be believed. The new index is a great help.

Finally, let us have a look at four space books. Closest to us in a physical sense is A.T. Lawton's *A Window in the Sky* (David and Charles: Newton Abbot, UK; £6.50). Lawton lifts the astronomical observer through the Earth's atmosphere and has written a most readable account of the achievements, possibilities and problems of observing from satellites. He discusses the astronomical objects that benefit most from this type of observation and the instrumentation required. The main emphasis is on the non-visual parts of the electromagnetic radiation spectrum, but even so I was rather surprised about the omission of a chapter on the Space Telescope.

Passage to Space: The Shuttle Transportation System (William Morrow; New York; \$6.95) by Charles Coombs, and *The Space Shuttle: Its Story and How to Make a Flying Paper Model* (Lothrop, Lee and

Shepard; New York; \$6.50) by Frank Ross Jr, are both short, profusely illustrated, simple accounts of the USA's next step in space exploration and are both aimed at the younger reader. There is very little to choose between these books; they are both excellent introductions to the subject. However, if I must choose, I would buy Frank Ross's (incidentally, the model flies beautifully).

We end this review with a flight of fantasy. *The Handbook for Space Pioneers* (Westbridge Books/David and Charles: Newton Abbot, UK; Grosset and Dunlap: New York; £5.95) by L. Stephen Wolfe and Roy L. Wysack, discusses the activities of a hypothetical organisation known as GAIL, the Galactic Association of Intelligent Life. Starships have roamed the neighbouring regions of our Galaxy searching for habitable planets. The book introduces pioneers who fancy leaving Earth to the physical characteristics and state of development of eight new worlds and it also provides a brief review of the starships that will be used to transport these emigrating Earth citizens. This book should appeal to all space flight enthusiasts and science fiction fans. The fun the authors had in producing it sparkles from every page.

David W. Hughes

David W. Hughes is Lecturer in Astronomy and Physics at the University of Sheffield, UK.



Illustration taken from *The End of the Game*, reviewed on page 640 of this issue.